CHAPTER IV

EXPERIMENT II

EFFECTS OF DIETARY MELAMINE, UREA-FORMALDEHYDE OR THEIR MIXTURES ON PERFORMANCE, CARCASS QUALITY, MELAMINE RESIDUES, MICROSCOPIC AND HISTOPATHOLOGICAL CHANGES IN BROILER TISSUES

4.1 Introduction

Melamine (1,3,5-triazine-2,4,6-triamine) has varied with widespread utilization by legitimate industry (Ingelfinger, 2008). It is also a metabolite of cyromazine in plants under certain environmental conditions (Lori et al., 1990). Because melamine contains 66% nitrogen by mass, it has been used as a non-protein nitrogen source for ruminants. However, Newton and Utley (1978) found that melamine did not serve as a satisfactory nitrogen supplement for cattle because its hydrolysis in cattle is slower and less complete than urea. Recently, reports indicating that pet food contaminated with melamine caused renal disease and deaths in cats and dogs have placed melamine contamination in the spotlight (Thomas and Kulkarni, 2007; Burns, 2007). In the case of pet food, it has been speculated that melamine was added intentionally in feed for a false high level of crude protein determined by the Kjeldahl method (Lachenmeier et al., 2009). Some swine, fish, and poultry feeds were reported to be contaminated with 30-120 mg of melamine/kg of feed (Burns, 2007; Nestle and Nesheim, 2007; Ingelfinger, 2008). Milk products and eggs contaminated with melamine were reported in Hong Kong and mainland China in 2008, which was considered to be a result of the illegal addition of melamine in milk or feed. Both melamine and formaldehyde or urea-formaldehyde (UF) are known as human health threats, and melamine-formaldehyde releases monomers of both (Ishiwata et al., 1986; Bradley et al., 2005). Urea-formaldehyde resin is the more important type of pelleting binders in both animal and aquatic feeds. Chicken diets are often pelleted to improve gains or feed utilization (Lanson and Smyth, 1955; Proudfoot and Hulan, 1980). Stilborn et al. (1991) was conducted to evaluate the effect of a urea-formaldehyde resin pellet binder on performance and blood parameters of laying hens over an eight-week feeding period. The laying hen was used as a model for all poultry species. The binder was incorporated into a nutritionally complete layer diet at 0, 0.2, and 0.4%. No significant effect was seen on performance and also no adverse effect was noted on blood parameters. The results of these studies indicate that urea-formaldehyde resin pellet binder can be used in poultry diets with no adverse effects on performance and blood parameters.

Documented research results pertaining to melamine in the production of animal feed are limited (Cruywagon et al., 2009). Clark (1966) reported that an intake of >10g/d caused in crystalluria in sheep and Mackenzie (1966) also reported weight loss and mortalities when melamine was fed to sheep. Brand et al. (2009) fed graded levels of melamine (0.50-3.00%) to young turkeys poults diets showed significant mortality was observed in turkeys fed 1.50, 2.00, 2.50 and 3.00% melamine with 27, 63, 93, and 93% mortality, respectively. Due to the high mortality in birds fed > 2.00% melamine, growth performance could only be evaluated in birds fed 0, 0.5, 1.00, and 1.50% melamine. Liver weight was not affected but kidney weight was higher when fed ≥ 1.00% melamine and showed pale and enlarged kidneys in turkeys fed 2.00-3.00% melamine. The bile of turkeys that died in these treatment groups contained crystals that were either microscopic (<2 micron) in size or were large white crystals visible to the naked eye. Ledoux et al. (2009) also fed graded levels of melamine in young broilers from hatch to 14 days indicated that feed intake was reduced in chickens fed diets containing ≥ 1.50% melamine, BWG decreased when fed ≥ 1.00% melamine with the greatest decrease occurring when fed ≥ 2.00% melamine, feed efficiency in converting feed to gain was less efficient when fed 2.50 and 3.00% melamine and also death was observed in chickens fed 2.50 and 3.00% melamine as early as day 5 of the study. Birds fed 2.50 and 3.00% melamine were fed less efficiently. Gross and microscopic examination in liver revealed crystals having enlarged pale kidneys and gallbladders containing an opaque bile. Lu et al. (2009a) fed graded levels of melamine (5-1,000 mg melamine /kg of diets) in broilers throughout the 42-d period showed no effects on weight gain, feed intake, feed conversion ratio and mortality of broiler chickens. Residue level of melamine in broiler tissues at d 28 and d 42 were below the detection limit when the diets contained ≤ 50 mg of melamine /kg of diet, but melamine was detected in breast meat and liver only in birds fed diet containing 500 and 1000 mg of melamine /kg of diet. Melamine distribution in different tissues was varied with the highest concentrations occurring in the kidneys. A withdrawal period of 7-d was found to clear melamine from the tissues. Lu et al. (2009b) reported that Cherry Valley ducks fed ≤ 1,000 mg/kg of the diet from d1 to d42 showed no difference in weight gain. On d42, melamine levels in breast meat, liver and kidney increased linearly with the increasing levels of melamine in diets containing more than 50 mg/kg melamine. The kidneys were found to accumulate the highest concentration of melamine. Gao et al. (2010) fed graded levels of melamine (1-100 ppm of diets) in laying ducts for 21 days followed by a 21 day withdrawal period indicated that dietary melamine had no adverse effects on laying performance. Renal lesions and melamine residue in eggs correlated with increasing levels of dietary melamine. The depletion time for melamine residue in eggs increased in parallel with the dietary melamine level. Bai et al. (2010) examined kidney samples from laying hens administrated with melamine at 8.60-140.90 mg/kg of BW/d for 34 days. The crystals were found in one of three kidneys of hens treated with melamine at either 62.60 or 140.90 mg/kg. In one recent study, Reimschuessel et al. (2008) suggested that chickens fed only melamine could develop spherulite crystals containing uric acid, a normal excretion product of chickens. Previous studies have also shown that pigs, fish, cats, and rat fed melamine or cyanuric acid in a 1:1 ratio develop renal crystals composed of melamine-cyanurate (Puschner et al., 2007; Dobson et al., 2008; Reimschuessel et al., 2008).

There is no report on degradation of melamine in poultry, that melamine may be degraded to some extent (Cruywagen et al., 2009). Melamine and cyanuric acid are rapidly absorbed in monogastric animals and excreted via urine in their unchanged forms (WHO, 2008). The problem is that melamine combines with cyanuric acid on a 1:1 basis to form spoke-like melamine cyanurate crystals in aqueous solutions at pH 5.8 or below (He et al., 2008). The objective of this study was to assess the effects of graded levels of melamine or UF alone or in combination when fed to broiler chicks on growth performance, carcass quality and to examine any clinical changes of the organs.

4.2 Materials and methods

4.2.1 Melamine and urea-formaldehyde (UF)

Melamine (purity ≥ 99.5%) was obtained from Tianjin BASF Chemical Company, Tianjin, China and UF resin (water content ≤ 2%, pH 7.0-7.5) was obtained from Luyuan Adhesive Material Co. LTD. Guangdong, China. Melamine and UF was diluted with ground corn to a concentration of 10% and appropriate amounts of this diluted material were then mixed in basal diets (Table 4.1) to prepare treatment diets with graded levels of melamine or UF or their equal mixtures.

4.2.2 Birds, feeding and management

The experimental procedures were reviewed and approved by the Adviser Committee of the Animal Science Department, Faculty of Agriculture, Khon Kaen University and animals were handled and managed according to the Guidelines of Experimental Animal Care from the National Research Council of Thailand. The study was conducted as a completely randomized design to evaluate the effects of graded levels of melamine or UF or mixture of melamine and UF (1:1) in broilers. Thirteen treatment diets were formulated to contain four graded levels (0.25, 0.50, 0.75 and 1.00%) of melamine, UF or their mixtures and no added for control.

One thousand and forty 1-d-old Arber Acres broiler chicken were randomly assigned to 52 floor pens (20 chicks per pen). Both male and female chicken were mixed in 50:50 ratio. The 13 dietary treatments were then randomly assigned to 4 replicate pens each.

A 4-phase feeding program (starter 1, starter 2, grower, and finisher) was used. The starter 1, starter 2, grower, and finisher diets were offered from d1 to 14, 15 to 28, 29 to 35, and 36 to 42, respectively. From d 43 to 49 all birds were fed a withdrawal diet that contained no melamine or UF or mixture of both. The diets were formulated based on corn, soybean meal and full fat soybean to meet standard industry specification (Table 4.1). The diets were fed in mash form. Feed was provided *ad* *libitum* and water was freely available throughout the study. Bird management followed the Arber Acres broiler management manual (Arber Acres, 2001). Birds were observed thrice daily for any signs of ill health or behavioral changes, and mortality was recorded daily.

Table 4.1 Composition of the basal diets (as-fed basis)

Ingredient , %	Starter 1 1-14d	Starter 2 15-28d	Grower 29-35d	Finisher 36-49d
Corn	47.85	52.66	58.05	58.03
Full fat soybean	20.00	20.00	17.00	20.50
Soybean meal	25.70	20.50	18.12	15.00
Monocalcium Phosphate	2.42	2.26	2.00	1.80
Limestone	1.70	1.80	1.60	1.60
DL-Methionine	0.31	0.22	0.18	0.17
L-Lysine	0.17	0.21	0.20	0.15
Rice bran oil (crude)	1.00	1.50	2.00	2.00
Salts	0.40	0.40	0.40	0.40
Choline chloride 50%	0.10	0.10	0.10	0.10
Vitamin-mineral premix[1]	0.25	0.25	0.25	0.25
Antibiotic premix[2]	0.10	0.10	0.10	-
Total	100.00	100.00	100.00	100.00
Calculated analysis:				
CP, %	23.05	20.18	18.45	18.33
ME, kcal/kg	3,069	3,122	3,172	3,212
Ca, %	1.05	1.06	1.02	0.98
Available P, %	0.48	0.46	0.44	0.43

[1]Supplied per kilogram of diet: vitamin A, 11,025 IU; vitamin D_3, 3,528 IU; vitamin E, 33 IU; K_3, 0.91 mg; thiamin, 2 mg; vitamin B_1, 18 mg; vitamin B_2, 8 mg; nicotinic acid, 55 mg; pantothenic acid, 18 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.028 mg; folic acid, 1 mg; biotin, 0.221 mg; manganese, 64 mg; iodine, 2 mg; zinc, 75 mg; iron, 40 mg; copper, 10 mg; selenium, 0.3 mg; choline, 478 mg.
[2]Supplied per kilogram of diet: salinomycin 60 mg.

4.2.3 Measurements and analysis

4.2.3.1 Growth performance

Chicken body weight (BW) and feed intake (FI) were recorded each week to calculate body weight gain (BWG) and feed conversion ratio (feed intake/weight gain, FCR).

4.2.3.2 Microscopic characterization of crystals in kidney, liver and spleen

On d 28, and 42, one chick was randomly selected from each replicate pen and euthanized by cervical dislocation, then samples of the breast meat, liver, kidney and spleen obtained and were kept at -20°C until analyzed by using an inverted microscope fitted with a digital video camera.

4.2.3.3 Carcass yield percentage

At d 42, after weighing, 12 birds per treatment were randomly selected and killed by cervical dislocation, the gastrointestinal tract was removed and weighed. Afterward, the birds were scalded, de-feathered, and eviscerated (with head, neck, blood, and hocks removed). Carcasses were weighed prior to deboning. Breast, thigh, drumstick, leg and wing were removed from each carcass and weighed for total carcass yield. The data on weight of edible tissues were recorded and all of the data were expressed as percentage of the pre-slaughter weight of the same bird.

4.2.3.4 Melamine analysis

Tissue samples (5 g) were extracted according to Lu et al. (2009). The melamine concentrations were determined using HPLC according the procedure outlined by Anderson et al. (2008). The detection limit of the assay for melamine was 2 mg/kg. Because UF resin pellet binder appears to be safe to use in broiler diets (Stilborn et al., 1991), therefore the UF concentration were not determined in these studies.

4.2.3.5 Histological examination

After sacrifice, liver, kidney and spleen of each animal were taken for histopathological examination. Organ samples were fixed in 10% buffered neutral formalin, embedded in paraffin, cut on a microtome in slices 4-5 μm thick and stained with hematoxylin and eosine. Wet-mount sections of organs were immediately evaluated via light microscopy for presence of crystals.

4.2.3.6 Statistical analysis

All data were analyzed using the one-way analysis of variance (ANOVA) and means were compared by Duncan's multiple-range test (SAS, 1995). Treatment means were tested for linear and quadratic effects of graded levels of either melamine or UF or their mixtures. Single degree of freedom contracts were made among treatment means. The pen was the experimental unit for all measurements. Differences were considered significant when $P<0.05$. A nonlinear regression model was used among treatments.

4.3 Results

4.3.1 Nutritive values of dietary diets

Table 4.2 shows the analyzed nutritive composition of the diets used in the experiment. Apparently, all diets had similar contents of moisture, crude protein, ether extract, calcium, phosphorus, ash, crude fiber and gross energy. The analyzed nutritive compositions of the diets were reasonable agreed with the calculated values.

Table 4.2 Analyzed nutritive composition of the experimental diets, during 1-14d, 15-28d, 29-35d and 36-42d of age

Composition	Starter I (1-14d)	Starter II (15-28d)	Grower (29-35d)	Finisher (36-42d)
Moisture, %	9.64	9.23	9.93	10.03
Crude protein, %	23.37	21.41	19.66	19.15
Ether extract, %	7.86	8.01	8.43	7.57
Calcium, %	1.19	1.19	1.00	1.00
Phosphorous, %	1.03	0.95	0.96	1.01
Ash, %	7.02	7.05	6.07	6.47
Fiber, %	3.27	3.15	3.07	3.18
Gross energy (kcal/kg)	4,680	4,960	4,940	4,870

4.3.2 Effects of melamine, UF or their equal mixtures on growth performance of broilers

4.3.2.1 Feed intake (FI)

Table 4.3 described the effect of dietary melamine, UF or their equal mixtures on FI of broilers during 1-14d and 15-28d of age. It was found that FI of all groups on both periods were significantly different (*P*<0.05). On 1-14d, FI of broilers ranged from 0.364 to 0.451 kg/h. Supplementation of melamine, UF or their equal mixtures at all levels showed decrease FI lower than control groups. The control group had the highest FI (0.451 kg/h). On 15-28d, FI ranged from 1.101 to 1.358 kg/h, combination of melamine and UF at level 1.00% showed the highest FI (1.358 kg/h).

Table 4.4 described the effect of dietary melamine, UF or their equal mixtures on FI of broilers during 29-35d, 36-42 and 1-42d of age. It was found that FI of all groups during 29-35d were significantly different (*P*<0.05). It ranged from 0.786 to 0.968 kg/h. However, FI of all groups during 36-42d and overall of age were not significantly different (*P*>0.05). On 36-42d, FI ranged from 1.040 to 1.254 kg/h, and overall periods 3.607 to 3.889 kg/h.

Table 4.3 Effects of melamine (M), urea-formaldehyde (UF) or their equal mixtures on feed intake of broilers from 1-14d and 15-28d of age

Treatment		Feed intake (kg per head)			
product	Concentration (%)	1-14d	RFI (%)	15-28d	RFI (%)
Basal	0	0.451[a]	100.00	1.316[ab]	100.00
M	0.25	0.389[bcd]	86.25	1.297[abc]	98.56
M	0.50	0.402[abcd]	89.14	1.253[abc]	95.21
M	0.75	0.364[d]	80.71	1.324[ab]	100.61
M	1.00	0.377[cd]	83.59	1.288[abc]	97.87
UF	0.25	0.391[bcd]	86.70	1.171[abc]	88.98
UF	0.50	0.388[bcd]	86.03	1.101[c]	83.66
UF	0.75	0.393[bcd]	87.14	1.335[ab]	101.44
UF	1.00	0.369[d]	81.82	1.150[bc]	87.39
M+UF	0.25	0.435[ab]	96.45	1.210[abc]	91.95
M+UF	0.50	0.422[abc]	93.57	1.275[abc]	96.88
M+UF	0.75	0.401[bcd]	88.91	1.307[ab]	99.32
M+UF	1.00	0.434[ab]	96.23	1.358[a]	103.19
Pooled SEM		0.015		0.061	
		----------------------Probability-------------------			
Product (P)		*		*	
Concentration (C)		NS		NS	
P x C		NS		NS	
Contrast					
Control vs other		**		NS	
L^1		NS		NS	
Q^1		*		NS	
L^2		NS		NS	
Q^2		NS		NS	
L^3		NS		NS	
Q^3		*		NS	

[a-d] Means within a column with no common superscript differ significant (*P*<0.05).

RFI= Relative feed intake index (%) = $\frac{\text{Feed intake (kg/h) of test group x 100}}{\text{Feed intake (kg/h) of control group}}$

L^1=Linear for M., Q^1=Quadratic for M, L^2=Linear for UF, Q^2=Quadratic for UF.
L^3=Linear for M + UF, Q^3=Quadratic for M + UF.
NS=Not significant; * *P*<0.05; ** *P*<0.01.

Table 4.4 Effect of melamine (M), urea-formaldehyde (UF) or their equal mixtures on feed intake of broilers from 29-35d, 36-42d and overall of age

Treatment		Feed intake (kg per head)					
product	Concentration (%)	29-35d	RFI (%)	36-42d	RFI (%)	1-42d	RFI (%)
Basal	0	0.922[ab]	100.00	1.052	100.00	3.740	100.00
M	0.25	0.888[ab]	96.31	1.113	105.80	3.701	98.96
M	0.50	0.932[ab]	101.08	1.135	107.89	3.735	99.87
M	0.75	0.968[a]	104.99	1.129	107.32	3.785	101.20
M	1.00	0.826[ab]	89.59	1.129	107.32	3.620	96.79
UF	0.25	0.786[b]	85.25	1.254	119.20	3.672	98.18
UF	0.50	0.912[ab]	98.92	1.206	114.64	3.855	103.07
UF	0.75	0.904[ab]	98.05	1.224	116.35	3.619	96.76
UF	1.00	0.952[ab]	103.25	1.188	112.93	3.704	99.04
M+UF	0.25	0.877[ab]	95.12	1.059	100.67	3.607	96.44
M+UF	0.50	0.914[ab]	99.13	1.049	99.71	3.753	100.35
M+UF	0.75	0.969[a]	105.10	1.213	115.30	3.889	103.98
M+UF	1.00	0.912[ab]	98.92	1.040	98.96	3.745	100.13
Pooled SEM		0.051		0.073		0.125	
		-------------------------------Probability-----------------------					
Product (P)		*		NS		NS	
Concentration (C)		NS		NS		NS	
P x C		NS		NS		NS	
Contrast							
Control vs other		*		NS		NS	
L^1		NS		NS		NS	
Q^1		NS		NS		NS	
L^2		NS		NS		NS	
Q^2		NS		NS		NS	
L^3		NS		NS		NS	
Q^3		NS		NS		NS	

[a-d] Means within a column with no common superscript differ significant (*P*<0.05).

RFI= Relative feed intake index (%) = $\frac{\text{Feed intake (kg/h) of test group} \times 100}{\text{Feed intake (kg/h) of control group}}$

L^1=Linear for M., Q^1=Quadratic for M, L^2=Linear for UF, Q^2=Quadratic for UF.
L^3=Linear for M + UF, Q^3=Quadratic for M + UF.
NS=Not significant; * *P*<0.05; ** *P*<0.01.

4.3.2.2 Body weight gain (BWG)

Table 4.5 described the effect of dietary melamine, UF or their mixtures on BWG of broilers during 1-14d and 15-28d of age. During 1-14d and 15-28d, it was found that BWG of all groups was significantly different ($P<0.05$). BWG of broiler receiving control diet during two periods showed the highest, 0.275 and 0.761 kg/h, respectively. BWG decreased in broiler fed melamine, UF or their equal mixture, with the greatest decreased in BWG observed in broilers fed 1.00% melamine of both two periods.

Table 4.6 described the effect of dietary melamine, UF or their equal mixtures on BWG of broilers during 29-35d, 36-42d and 1-42d of age. BWG decreased significantly different ($P<0.05$) of all groups but not significantly during 36-42d of age. However, broiler fed melamine or UF or their mixtures showed trend decreased BWG when compared with control groups. Overall periods of age (1-42d), it was found that BWG of broilers fed control diet had the highest BWG, 1.967 kg/h, while BWG of broilers fed 1.00% melamine was lower than of the other groups, 1.611 kg/h and had relative growth index of 81.90%. Significant linear ($P<0.05$) affected on BWG as the levels of melamine in the diets increased. There was no significant difference ($P>0.05$) in BWG between broilers fed diets containing UF or their equal mixtures compared with controls.

Table 4.5 Effects of melamine (M), urea-formaldehyde (UF) or their equal mixtures on body weight gain of broilers from 1-14d and 15-28d of age

Treatment		Body weight gain (kg per head)			
product	Concentration (%)	1-14d	RGI (%)	15-28d	RGI (%)
Basal	0	0.275[a]	100.00	0.761[a]	100.00
M	0.25	0.237[bcd]	86.18	0.696[abc]	91.46
M	0.50	0.230[cd]	83.63	0.607[cd]	79.76
M	0.75	0.218[d]	79.27	0.618[cd]	81.21
M	1.00	0.212[d]	77.09	0.558[d]	73.32
UF	0.25	0.241[abcd]	87.64	0.630[cd]	82.79
UF	0.50	0.242[abcd]	88.00	0.617[cd]	81.08
UF	0.75	0.238[bcd]	86.55	0.678[abc]	89.09
UF	1.00	0.233[cd]	84.73	0.632[cd]	83.05
M+UF	0.25	0.277[a]	100.73	0.648[bcd]	85.15
M+UF	0.50	0.257[abc]	93.45	0.745[ab]	97.90
M+UF	0.75	0.249[abcd]	90.55	0.707[abc]	92.90
M+UF	1.00	0.272[ab]	98.91	0.744[ab]	97.77
Pooled SEM		0.011		0.034	
		----------------------Probability--------------------			
Product (P)		**		**	
Concentration (C)		NS		NS	
P x C		NS		NS	
Contrast					
Control vs other		*		*	
L^1		NS		NS	
Q^1		**		**	
L^2		NS		NS	
Q^2		NS		NS	
L^3		NS		*	
Q^3		NS		NS	

[a-d] Means within a column with no common superscript differ significant (P<0.05).

RGI= Relative growth index (%) = $\frac{\text{Body weight gain (kg/h) of test group x 100}}{\text{Body weight gain (kg/h) of control group}}$

L^1=Linear for M., Q^1=Quadratic for M, L^2=Linear for UF, Q^2=Quadratic for UF.
L^3=Linear for M + UF, Q^3=Quadratic for M + UF.
NS=Not significant; * P<0.05; ** P<0.01.

Table 4.6 Effects of melamine (M), urea-formaldehyde (UF) or their equal mixtures on body weight gain of broilers from 29-35d, 36-42d and overall of age

Treatment		Body weight gain (kg per head)					
product	Concentration (%)	29-35d	RGI (%)	36-42d	RGI (%)	1-42d	RGI (%)
Basal	0	0.464[a]	100.00	0.468	100.00	1.967[a]	100.00
M	0.25	0.373[ab]	80.39	0.430	91.88	1.736[ab]	88.26
M	0.50	0.417[ab]	89.87	0.455	97.22	1.739[ab]	88.54
M	0.75	0.435[ab]	93.75	0.474	101.28	1.745[ab]	88.71
M	1.00	0.406[ab]	87.50	0.435	92.95	1.611[b]	81.90
UF	0.25	0.312[b]	67.24	0.373	79.70	1.786[ab]	90.80
UF	0.50	0.405[ab]	87.28	0.392	83.76	1.769[ab]	89.93
UF	0.75	0.419[ab]	90.30	0.433	92.52	1.668[ab]	84.80
UF	1.00	0.445[a]	95.91	0.454	97.01	1.693[ab]	86.07
M+UF	0.25	0.433[ab]	93.32	0.427	91.24	1.807[ab]	91.87
M+UF	0.50	0.444[a]	95.69	0.454	97.01	1.899[ab]	96.54
M+UF	0.75	0.479[a]	103.23	0.393	83.97	1.839[ab]	93.49
M+UF	1.00	0.452[a]	97.41	0.439	93.80	1.906[ab]	96.90
Pooled SEM		0.038		0.036		0.090	
		------------------------------Probability-----------------------					
Product (P)		*		NS		*	
Concentration (C)		NS		NS		NS	
P x C		NS		NS		NS	
Contrast							
Control vs other		*		NS		**	
L^1		NS		NS		**	
Q^1		NS		NS		NS	
L^2		NS		NS		NS	
Q^2		NS		NS		NS	
L^3		*		NS		NS	
Q^3		NS		NS		NS	

[a-d] Means within a column with no common superscript differ significant (*P*<0.05).
RGI= Relative growth index (%) = $\frac{\text{Body weight gain (kg/h) of test group x 100}}{\text{Body weight gain (kg/h) of control group}}$
L^1=Linear for M., Q^1=Quadratic for M, L^2=Linear for UF, Q^2=Quadratic for UF.
L^3=Linear for M + UF, Q^3=Quadratic for M + UF.
NS=Not significant; * *P*<0.05; ** *P*<0.01.

4.3.2.3 Feed conversion ratio (FCR)

Table 4.7 described the effect of dietary melamine, UF or their equal mixtures on FCR of broilers during 1-14d and 15-28d of age. It was found that FCR of all groups were significantly different (*P*<0.05) for both two periods. During 1-14d, broilers fed 1.00% melamine showed the highest FCR and had relative FCR index (RFCRI) -8.41%. Similar FCR also showed during 15-28d, broilers fed 1.00% melamine showed the highest FCR and had RFCRI decreasing feed efficiency -33.41% when compared with control groups.

Table 4.8 described the effect of dietary melamine, UF or their equal mixtures on FCR of broilers during 29-35d, 36-42d and 1-42d of age. FCR showed decrease feed efficiency significantly different (*P*<0.05) when compared with control groups of all periods of testing. During 1-42d, FCR showed the improved highest decreasing feed efficiency with broilers fed 1.00% melamine (2.247) and had RFCRI of -18.20% when compared with control groups showed the best feed efficiency (1.907). FCR showed less efficient linearly (*P*<0.05) with increasing levels of melamine in the diets and showed the greatest decreased efficiency when fed 1.00% melamine, but no such effect was observed for broilers fed UF or their equal mixtures.

Table 4.7 Effects of melamine (M), urea-formaldehyde (UF) or their equal mixtures on feed conversion ratio of broilers from 1-14d and 15-28d of age

Treatment		Feed conversion ratio			
product	Concentration (%)	1-14d	RFCRI (%)	15-28d	RFCRI (%)
Basal	0	1.640^{bc}	0.00	1.730^{d}	0.00
M	0.25	1.641^{bc}	-0.06	1.864^{bcd}	-7.74
M	0.50	1.747^{ab}	-6.52	2.064^{bc}	-19.31
M	0.75	1.669^{abc}	-1.77	2.142^{ab}	-23.81
M	1.00	1.778^{a}	-8.41	2.308^{a}	-33.41
UF	0.25	1.627^{bc}	+0.79	1.859^{bcd}	-7.45
UF	0.50	1.599^{bc}	+2.50	1.784^{cd}	-3.12
UF	0.75	1.651^{bc}	-0.67	1.969^{bcd}	-13.81
UF	1.00	1.583^{c}	+3.47	1.820^{bcd}	-5.20
M+UF	0.25	1.570^{c}	+4.27	1.867^{bcd}	-7.92
M+UF	0.50	1.642^{bc}	-0.12	1.711^{d}	+1.10
M+UF	0.75	1.610^{bc}	+1.83	1.848^{bcd}	-6.82
M+UF	1.00	1.601^{bc}	+2.38	1.825^{bcd}	-5.49
Pooled SEM		0.043		0.099	
		----------------------Probability-------------------			
Product (P)		*		**	
Concentration (C)		NS		NS	
P x C		NS		NS	
Contrast					
Control vs other		*		*	
L^1		NS		NS	
Q^1		NS		*	
L^2		NS		NS	
Q^2		NS		NS	
L^3		NS		NS	
Q^3		NS		NS	

[a-d] Means within a column with no common superscript differ significant (P<0.05).
RFCRI = Relative feed conversion ratio index (%)

$$= \left\{ \frac{\text{Feed conversion ratio of test group x 100}}{\text{Feed conversion ratio of control group}} \right\} - 100$$

L^1=Linear for M., Q^1=Quadratic for M, L^2=Linear for UF, Q^2=Quadratic for UF.
L^3=Linear for M + UF, Q^3=Quadratic for M + UF.
NS=Not significant; * P<0.05; ** P<0.01.

Table 4.8 Effects of melamine (M), urea-formaldehyde (UF) or their equal mixtures on feed conversion ratio of broilers from 29-35d, 36-42d and overall of age

Treatment		Feed conversion ratio					
product	Concentration (%)	29-35d	RFCRI (%)	36-42d	RFCRI (%)	1-42d	RFCRI (%)
Basal	0	1.987^{b}	0.00	2.247^{c}	0.00	1.901^{b}	0.00
M	0.25	2.380^{ab}	-19.78	2.588^{bc}	-15.18	2.131^{ab}	-12.09
M	0.50	2.235^{ab}	-12.48	2.494^{bc}	-10.99	2.147^{ab}	-12.94
M	0.75	2.225^{ab}	-11.97	2.381^{bc}	-5.96	2.168^{ab}	-14.04
M	1.00	2.034^{b}	-2.37	2.595^{bc}	-15.53	2.247^{a}	-18.20
UF	0.25	2.520^{a}	-26.82	3.361^{a}	-49.58	2.055^{ab}	-8.10
UF	0.50	2.251^{ab}	-13.29	3.076^{ab}	-36.89	2.180^{ab}	-14.68
UF	0.75	2.156^{ab}	-8.51	2.826^{abc}	-25.76	2.170^{ab}	-14.15
UF	1.00	2.138^{b}	-7.59	2.616^{bc}	-16.42	2.187^{ab}	-15.04
M+UF	0.25	2.025^{b}	-1.91	2.480^{bc}	-10.37	1.997^{ab}	-5.05
M+UF	0.50	2.058^{b}	-3.57	2.310^{bc}	-2.80	1.976^{ab}	-3.95
M+UF	0.75	2.023^{b}	-1.81	3.086^{ab}	-37.33	2.115^{ab}	-11.25
M+UF	1.00	2.017^{b}	-1.51	2.369^{bc}	-5.43	1.964^{ab}	-3.31
Pooled SEM		0.173		0.254		0.093	
		-------------------------------Probability------------------------					
Product (P)		*		*		**	
Concentration (C)		NS		NS		NS	
P x C		NS		NS		*	
Contrast							
Control vs other		*		*		**	
L^1		*		NS		*	
Q^1		NS		NS		NS	
L^2		NS		*		NS	
Q^2		NS		NS		NS	
L^3		*		*		NS	
Q^3		NS		NS		NS	

$^{a-d}$ Means within a column with no common superscript differ significant (P<0.05).

RFCRI = Relative feed conversion ratio index (%)

$$= \left\{ \frac{\text{Feed conversion ratio of test group x 100}}{\text{Feed conversion ratio of control group}} \right\} - 100$$

L^1=Linear for M., Q^1=Quadratic for M, L^2=Linear for UF, Q^2=Quadratic for UF.
L^3=Linear for M + UF, Q^3=Quadratic for M + UF.
NS=Not significant; * P<0.05; ** P<0.01.

4.3.2.4 Summary of growth performance

As previously report, dietary melamine, UF or their equal mixtures at all levels significantly decreased (*P*<0.05) growth performance such as BWG and FCR showed less efficiency than control groups (*P*<0.05) (Table 4.9). There were no visible signs of ill health or changes in the behavior of broilers during the 49-d experimental period. There was no difference (*P*>0.05) in FI among controls and chickens fed all graded levels of melamine, UF or their equal mixture. BWG decreased significantly (*P*<0.05) in birds fed ≥ 0.75% melamine, with the greatest decrease in BWG observed in birds fed 1.00% melamine. There was no significant difference (*P*>0.05) in BWG between birds fed diets containing UF or their equal mixtures compared with controls. There were significant linear (*P*<0.05) effects on BWG as the levels of melamine in the diets increased. No difference in FI among the controls and chickens fed 0.50 or 1.00% melamine. FCR was significantly less efficient (*P*<0.05) for birds fed either melamine, UF or their equal mixtures compared with the controls. FCR showed a less efficient linear (*P*<0.05) with increasing levels of melamine and showed the greatest decreased efficiency when fed 1.00% melamine, while no such effects were observed in birds fed UF or their equal mixtures.

The survival percentage significantly decreased (*P*<0.05) in birds fed either melamine, UF or their equal mixtures compared with the controls. Survival percentage did not differ (*P*>0.05) between birds fed diets containing UF or their equal mixtures. There was a significant quadratic (*P*<0.05) effect on the survival percentage as the levels of either melamine, UF or their equal mixtures in the diets increased. The survival percentage did not different (*P*>0.05) between birds fed either UF or their equal mixtures, but showed a greatest decrease when birds were fed on 1.00% melamine. Survival percentage also decreased (*P*<0.05) in broilers fed 1.00% UF and also 1.00% of their equal mixtures, but there was no difference (*P*>0.05) in BWG, FI and FCR compared with the controls. The Productive index (PI) or European productive efficiency factor (EPEF) significantly decreased (*P*<0.05) in birds fed either melamine or UF or their equal mixtures compared with the controls. The influence of melamine, UF or their equal mixtures of the PI showed economic lost significant linear (*P*<0.05) and quadratic (*P*<0.05) effects as the levels of either melamine, UF or their equal mixtures of the diets increased.

4.3.3 Effects of melamine, urea-formaldehyde (UF) or their equal mixtures on carcass quality

The carcass quality at termination, including percentage of carcass dressing, breast meat, thigh, drumstick, wing and abdominal fat. This experiment found that all treatment groups were not significantly different (P>0.05) as showed in Table 4.10. Feeding graded levels of melamine or UF alone or their equal mixture showed no difference (P>0.05) in carcass quality (dressing percentage, percentage of breast meat, drumstick, thigh, wing, leg and total carcass yield), abdominal fat weight and organs weight (heart, spleen and gizzard) except liver weight among control no added groups and chicks fed with melamine or UF alone or their equal mixture (Table 4.11). Dietary melamine, UF or their equal mixtures trended to decrease on both dressing percentage and total carcass yield. The liver weight of the treated groups were higher (P<0.05) than the control which found to be enlarged pale and edematous liver.

Table 4.9 Effects of melamine (M), urea-formaldehyde (UF) or their equal mixture on growth performance, survival rate and Productive index (PI) of broiler

Parameter	Treatment													Pooled SEM
	M, %				UF, %				M+PUF, %				Control	
	0.25	0.50	0.75	1.00	0.25	0.50	0.75	1.00	0.25	0.50	0.75	1.00		
1-14d:														
BWG, g	237^{bcd}	230^{cd}	218^{d}	212^{d}	241^{abcd}	242^{abcd}	238^{bcd}	233^{cd}	277^{a}	257^{abc}	249^{abcd}	272^{ab}	275^{a}	0.011
FI, g	389^{bcd}	402^{bcd}	364^{d}	377^{cd}	392^{bcd}	388^{bcd}	393^{bcd}	369^{d}	435^{ab}	422^{abc}	401^{bcd}	434^{ab}	451^{a}	0.015
FCR	1.641^{bc}	1.747^{ab}	1.669^{abc}	1.778^{a}	1.627^{bc}	1.599^{bc}	1.651^{bc}	1.583^{c}	1.570^{c}	1.642^{bc}	1.610^{bc}	1.601^{bc}	1.640^{bc}	0.043
Survival, %	97.90	96.85	98.95	97.90	98.95	98.95	98.95	100.00	97.90	98.95	98.95	98.95	97.90	1.063
15-28d:														
BWG, g	696^{abc}	607^{bcd}	618^{cd}	558^{d}	630^{abcd}	617^{cd}	678^{abc}	632^{abcd}	648^{abcd}	745^{ab}	707^{abc}	744^{ab}	761^{a}	0.034
FI, g	1297^{abc}	1253^{abc}	1324^{ab}	1288^{abc}	1171^{abc}	1101^{c}	1335^{ab}	1150^{bc}	1210^{abc}	1275^{abc}	1307^{ab}	1358^{a}	1316^{ab}	0.061
FCR	1.864^{bcd}	2.064^{bc}	2.142^{ab}	2.308^{a}	1.859^{bcd}	1.784^{cd}	1.969^{bcd}	1.820^{bcd}	1.867^{bcd}	1.711^{d}	1.848^{bcd}	1.825^{bcd}	1.730^{d}	0.099
Survival, %	95.80	95.78	95.78	94.70	95.83	96.85	94.75	95.80	97.88	96.85	94.75	96.83	97.90	1.275
29-35d:														
BWG, g	373^{ab}	417^{ab}	435^{ab}	406^{ab}	312^{b}	405^{ab}	419^{ab}	445^{a}	433^{ab}	444^{a}	479^{a}	452^{a}	464^{a}	0.038
FI, g	888^{ab}	932^{ab}	968^{a}	826^{ab}	786^{b}	912^{ab}	904^{ab}	952^{ab}	877^{ab}	914^{ab}	969^{ab}	912^{ab}	922^{ab}	0.051
FCR	2.380^{ab}	2.235^{ab}	2.225^{ab}	2.034^{b}	2.520^{a}	2.251^{ab}	2.156^{ab}	2.138^{b}	2.025^{b}	2.058^{b}	2.023^{b}	2.017^{b}	1.987^{b}	0.173
Survival, %	96.73^{ab}	97.75^{ab}	94.55^{b}	94.48^{b}	98.93^{ab}	97.85^{ab}	97.85^{ab}	97.80^{ab}	97.88^{ab}	97.85^{ab}	98.93^{ab}	95.78^{ab}	100.00^{a}	1.369
36-42d:														
BWG, g	430	455	474	435	373	392	433	454	427	454	393	439	468	0.036
FI, g	1113	1135	1129	1129	1254	1206	1224	1188	1059	1049	1213	1040	1052	0.073
FCR	2.588^{bc}	2.494^{bc}	2.381^{bc}	2.595^{bc}	3.361^{a}	3.076^{ab}	2.826^{abc}	2.616^{bc}	2.480^{bc}	2.310^{bc}	3.086^{ab}	2.369^{bc}	2.247^{c}	0.254
Survival, %	100.00^{a}	98.93^{a}	95.30^{b}	95.25^{b}	100.00^{a}	98.88^{a}	100.00^{a}	98.93^{a}	98.93^{a}	97.85^{ab}	98.88^{a}	97.75^{ab}	100.00^{a}	1.000
1-42d:														
BWG, g	1736^{ab}	1739^{ab}	1745^{ab}	1611^{b}	1786^{ab}	1769^{ab}	1668^{ab}	1693^{ab}	1807^{ab}	1899^{ab}	1839^{ab}	1906^{ab}	1967^{a}	0.090
FI, g	3701	3735	3785	3620	3672	3855	3619	3704	3607	3753	3889	3745	3740	0.125
FCR	2.131^{ab}	2.147^{ab}	2.168^{ab}	2.247^{a}	2.055^{ab}	2.180^{ab}	2.170^{ab}	2.187^{ab}	1.997^{ab}	1.976^{ab}	2.115^{ab}	1.964^{ab}	1.901^{b}	0.093
Survival, %	90.63^{b}	89.59^{bc}	85.42^{cd}	83.34^{d}	93.75^{ab}	92.7^{b}	91.67^{ab}	90.63^{b}	92.71^{ab}	91.67^{ab}	90.63^{b}	89.59^{bc}	95.83^{a}	1.405
PI*	176.36^{bc}	173.81^{bc}	164.68^{bc}	144.22^{c}	195.95^{abc}	180.00^{bc}	168.46^{bc}	168.96^{bc}	199.87^{ab}	210.09^{ab}	189.36^{abc}	207.65^{ab}	236.86^{a}	16.423

^{a,b,c,d,e}Means with different superscripts within the same row differ (P<0.05).

$$*PI = \frac{BWG \times Survival\ rate}{age\ (d) \times FCR \times 100}$$

Table 4.10 Effects of melamine (M), urea-formaldehyde (UF) or their equal mixtures on carcass quality at termination (42d)

Treatment		Carcass quality (%)						
product	Concentration (%)	Dressing percentage	Breast meat	drumstick	thigh	wing	leg	Total carcass yield
Basal	0	81.47	17.64	11.81	10.63	8.20	3.58	51.86
M	0.25	81.07	17.02	11.66	10.43	8.31	3.43	50.85
M	0.50	80.01	17.22	11.61	10.69	8.53	3.65	51.70
M	0.75	80.07	16.45	11.54	9.84	7.82	3.56	49.21
M	1.00	80.59	16.42	11.56	10.82	8.23	3.50	50.53
UF	0.25	81.40	18.11	11.49	10.19	8.19	3.40	51.38
UF	0.50	81.91	17.30	11.54	10.40	7.98	3.48	50.69
UF	0.75	82.03	17.43	11.59	10.38	7.97	3.26	50.63
UF	1.00	81.00	17.07	11.56	9.95	8.17	3.42	50.17
M+UF	0.25	81.72	16.87	11.61	10.75	8.09	3.41	50.73
M+UF	0.50	81.24	16.26	11.71	10.11	8.10	3.47	49.64
M+UF	0.75	81.08	16.74	11.66	10.46	8.43	3.46	50.75
M+UF	1.00	81.73	17.56	11.56	10.33	8.27	3.41	51.13
Pooled SEM		0.871	0.576	0.311	0.321	0.236	0.156	0.820
		-----------------------------Probability-----------------------						
Product (P)		NS	NS	NS	NS	NS	NS	NS
Concentration (C)		NS	NS	NS	NS	NS	NS	NS
P x C		NS	NS	NS	NS	NS	NS	NS
Contrast								
Control vs other		NS	NS	NS	NS	NS	NS	NS
L^1		NS	NS	NS	NS	NS	NS	NS
Q^1		NS	NS	NS	NS	NS	NS	NS
L^2		NS	NS	NS	NS	NS	NS	NS
Q^2		NS	NS	NS	NS	NS	NS	NS
L^3		NS	NS	NS	NS	NS	NS	NS
Q^3		NS	NS	NS	NS	NS	NS	NS

L^1=Linear for M., Q^1=Quadratic for M, L^2=Linear for UF, Q^2=Quadratic for UF.
L^3=Linear for M + UF, Q^3=Quadratic for M + UF.
NS=Not significant; * $P<0.05$; ** $P<0.01$.

Table 4.11 Effects of melamine (M), urea-formaldehyde (UF) or their equal mixtures on carcass quality at termination (42d)

Treatment		Carcass quality (%)				
Product	Concentration (%)	Liver	Heart	Spleen	Gizzard	Abdominal fat
Basal	0	1.67^{e}	0.46	0.09	1.39	1.41
M	0.25	1.77^{de}	0.42	0.11	1.44	1.58
M	0.50	1.87^{cde}	0.48	0.10	1.42	1.35
M	0.75	2.08^{abc}	0.48	0.10	1.51	1.31
M	1.00	2.29^{a}	0.49	0.11	1.46	1.52
UF	0.25	1.94^{bcde}	0.47	0.10	1.52	1.51
UF	0.50	1.90^{cde}	0.47	0.10	1.43	1.51
UF	0.75	1.97^{bcd}	0.47	0.10	1.39	1.43
UF	1.00	2.22^{ab}	0.49	0.11	1.41	1.51
M+UF	0.25	1.94^{bcde}	0.47	0.10	1.46	1.50
M+UF	0.50	2.02^{abcd}	0.49	0.10	1.51	1.44
M+UF	0.75	2.07^{abcd}	0.45	0.10	1.47	1.45
M+UF	1.00	2.09^{abc}	0.46	0.10	1.47	1.49
Pooled SEM		0.091	0.024	0.006	0.049	0.099
		--------------------------------Probability------------------------				
Product (P)		*	NS	NS	NS	NS
Concentration (C)		NS	NS	NS	NS	NS
P x C		NS	NS	NS	NS	NS
Contrast						
Control vs other		NS	NS	NS	NS	NS
L^1		NS	NS	NS	NS	NS
Q^1		NS	NS	NS	NS	NS
L^2		NS	NS	NS	NS	NS
Q^2		NS	NS	NS	NS	NS
L^3		NS	NS	NS	NS	NS
Q^3		NS	NS	NS	NS	NS

[a-e] Means within a column with no common superscript differ significant ($P<0.05$), L^1=Linear for M., Q^1=Quadratic for M, L^2=Linear for UF, Q^2=Quadratic for UF. L^3=Linear for M + UF, Q^3=Quadratic for M + UF.
NS=Not significant; * $P<0.05$; ** $P<0.01$.

4.3.4 Effects of melamine (M) or urea-formaldehyde (UF) or their equal mixtures on composition of breast meat of broilers at termination

The composition of breast meat of broilers at termination, including percentage of dry matter, crude protein and fat were shown in Table 4.12. It was found that broilers fed melamine or UF or their equal mixtures were not significantly different (P>0.05) on composition of breast meat. Dry matter of breast meat ranging from 27.03 to 28.78%, crude protein 22.17 to 23.57% and fat by ether extract 2.33 to 2.73%.

Table 4.12 Effects of melamine (M) or urea-formaldehyde (UF) or their equal mixtures on composition of breast meat of broilers at termination

Treatment		Composition of breast meat (%)		
Product	Concentration (%)	Dry matter	Crude protein	Fat
Basal	0	27.49	22.83	2.48
M	0.25	28.00	22.37	2.73
M	0.50	28.49	23.06	2.71
M	0.75	28.51	23.28	2.65
M	1.00	28.79	23.57	2.33
UF	0.25	27.03	22.17	2.69
UF	0.50	28.62	23.35	2.44
UF	0.75	28.50	23.49	2.33
UF	1.00	27.62	22.45	2.35
M+UF	0.25	28.12	23.31	2.43
M+UF	0.50	28.59	23.30	2.33
M+UF	0.75	28.40	23.45	2.35
M+UF	1.00	27.46	22.62	2.45
Pooled SEM		0.399	0.666	0.128
		-------------------------Probability-------------		
Product (P)		NS	NS	NS
Concentration (C)		NS	NS	NS
P x C		NS	NS	NS
Contrast				
Control vs other		NS	NS	NS
L^1		NS	NS	NS
Q^1		NS	NS	NS
L^2		NS	NS	NS
Q^2		NS	NS	NS
L^3		NS	NS	NS
Q^3		NS	NS	NS

L^1=Linear for M., Q^1=Quadratic for M, L^2=Linear for UF, Q^2=Quadratic for UF.
L^3=Linear for M + UF, Q^3=Quadratic for M + UF.
NS=Not significant; * $P<0.05$; ** $P<0.01$.

4.3.5 Residual melamine concentration in tissues

Residual melamine concentration in breast meat and liver on d42 and d49 are summarized in Table 4.13. The residue levels of melamine in breast meat and liver at d42 were below the detection when the diets contained ≤ 0.50% melamine supplementation in the diet. At d42, melamine levels increased ($P<0.05$) in both breast meat and liver with the increasing levels of melamine in the diets. A similar trend was observed in both breast meat and liver in birds fed with their equal mixtures, but melamine was detected in breast meat and liver only in birds fed diet containing their equal mixtures 0.75 and 1.00% in the diet. The distribution of residual melamine in the liver was higher ($P<0.05$) than in the breast meat. After a 7d withdrawal period, residual melamine was not detected in both breast meat and liver.

Table 4.13 Concentration of melamine (M) residues in breast meat and liver fed diets containing graded levels of two products (mg/kg of dry weight)[1,2]

Treatment		Day 42		Day 49	
Product	Added (%)	Breast meat	Liver	Breast meat	Liver
M	0.25	2.69^{d}	2.92^{e}	ND[2]	ND
M	0.50	4.46^{c}	4.86^{c}	ND	ND
M	0.75	$7.34^{b,y}$	$9.48^{b,x}$	ND	ND
M	1.00	$11.91^{a,y}$	$13.15^{a,x}$	ND	ND
M+UF	0.25	0.83^{f}	1.01^{g}	ND	ND
M+UF	0.50	1.89^{e}	1.98^{f}	ND	ND
M+UF	0.75	3.76^{c}	3.97^{d}	ND	ND
M+UF	1.00	4.22^{c}	4.52^{cd}	ND	ND
Pooled SEM		0.2533	0.2314		

$^{a-f}$ Means in a column within no common superscripts differ significant ($P<0.05$).
^{x,y} For each tissue, means in a raw within no common superscripts differ significant ($P<0.05$).
[1] Each value represents the mean ± SD of tissue sample from 4 birds.
[2] Not detect; the detect limit of the assay was 2 mg of M/kg of tissue dry matter. Average values of less than 2 mg/kg indicate that melamine was not detected in some samples, thus lowering the average value below 2 mg/kg of tissue dry matter.

4.3.6 Microscopic examination of crystal in various tissues

Melamine “crystals” were detected in the liver, spleen and kidney of birds subjected to graded levels of melamine from 0.25 to 1.00% in the diets. Compared with the controls (Figure 4.1), obvious golden-brown or pale yellow crystals with “spoke wheel” appearance were seen in the tissues of the liver, kidney and spleen of the treated birds. The crystals in the tissues of birds fed with 0.50% melamine in their diet was < 2 micron and some were large enough to be visible to the naked eye. Microscopic examination of the melamine “crystals” in the liver, kidney and spleen fed with graded levels of melamine (0, 0.25, 0.50, 0.75 and 1.00% melamine in the diets) were presented in Figure 4.1. The liver, kidney and spleen tissues examined under microscopy revealed “golden-brown crystals” have a globular to flattened shape with fine linear radiations. For the birds fed control diets, there were no obvious changes in the tissues structure and golden-brown crystals in the liver, kidney and spleen tissues (Figure 1A, 2A, 3A). The amount and the size of golden-brown crystals of melamine and also tissue lesions become more score and evident with increasing dietary melamine concentration (Figure B1-B3, C1-C3, D1-D3, E1-E3)

4.3.7 Histological examination of crystal in various tissues

No pathological change was observed in the tissue organs of broilers fed with negative control. Histological lesions were observed in the kidney, liver and spleen fed with graded levels of melamine (0, 0.25, 0.50, 0.75 and 1.00% melamine in the diets) compared with control group, there were no obvious changes in the kidney, liver and spleen structure of broilers fed $\leq$0.50% of melamine. The kidney, liver and spleen lesions became more severe with increasing dietary melamine concentration. Liver tissues showed hepatocyte swelling, congestion, cell membrane less prominent, red blood cell distension and sinusoidal dilatation. Spleen tissues showed congestion and white blood cell infiltration. Melamine crystals were not found in the livers and spleens of any broilers. Photomicrograph showed brown melamine crystals deposited in the interstitial tissue surrounding the renal tubules of laying hens fed 1.00% melamine contaminated in diet (Figure 4.2).

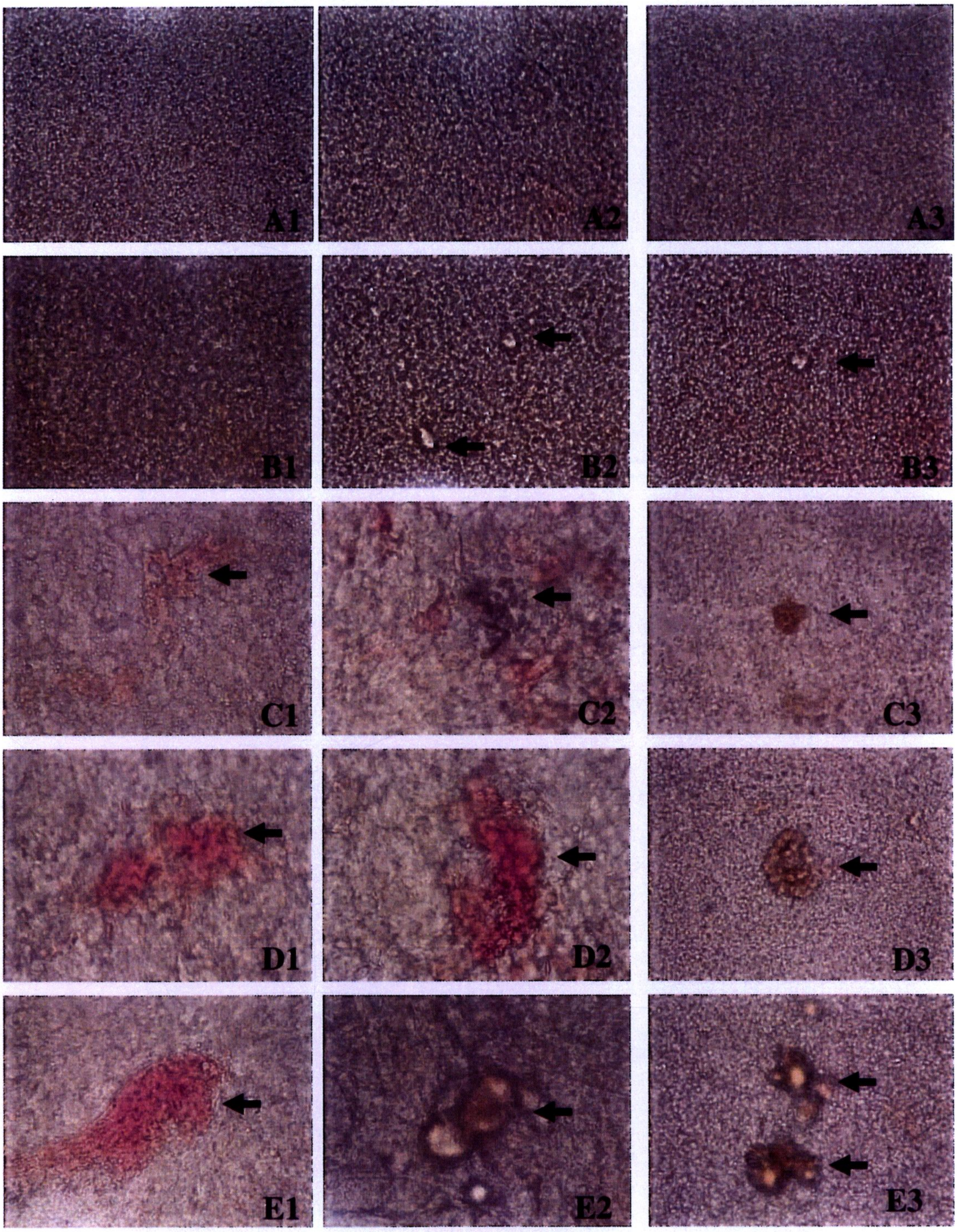

Figure 4.1 A microscopic view of the graded levels of dietary melamine supplementation in broiler diets for 42 days in the tissues of the liver (A1-E1), kidney (A2-E2) and spleen (A3-E3) with magnification 400-1000X under inverted microscopy: (A) control; (B) 0.25% melamine in the diet; (C) 0.50% melamine in the diet; (D) 0.75% melamine in the diet; (E) 1.00% melamine in the diet.

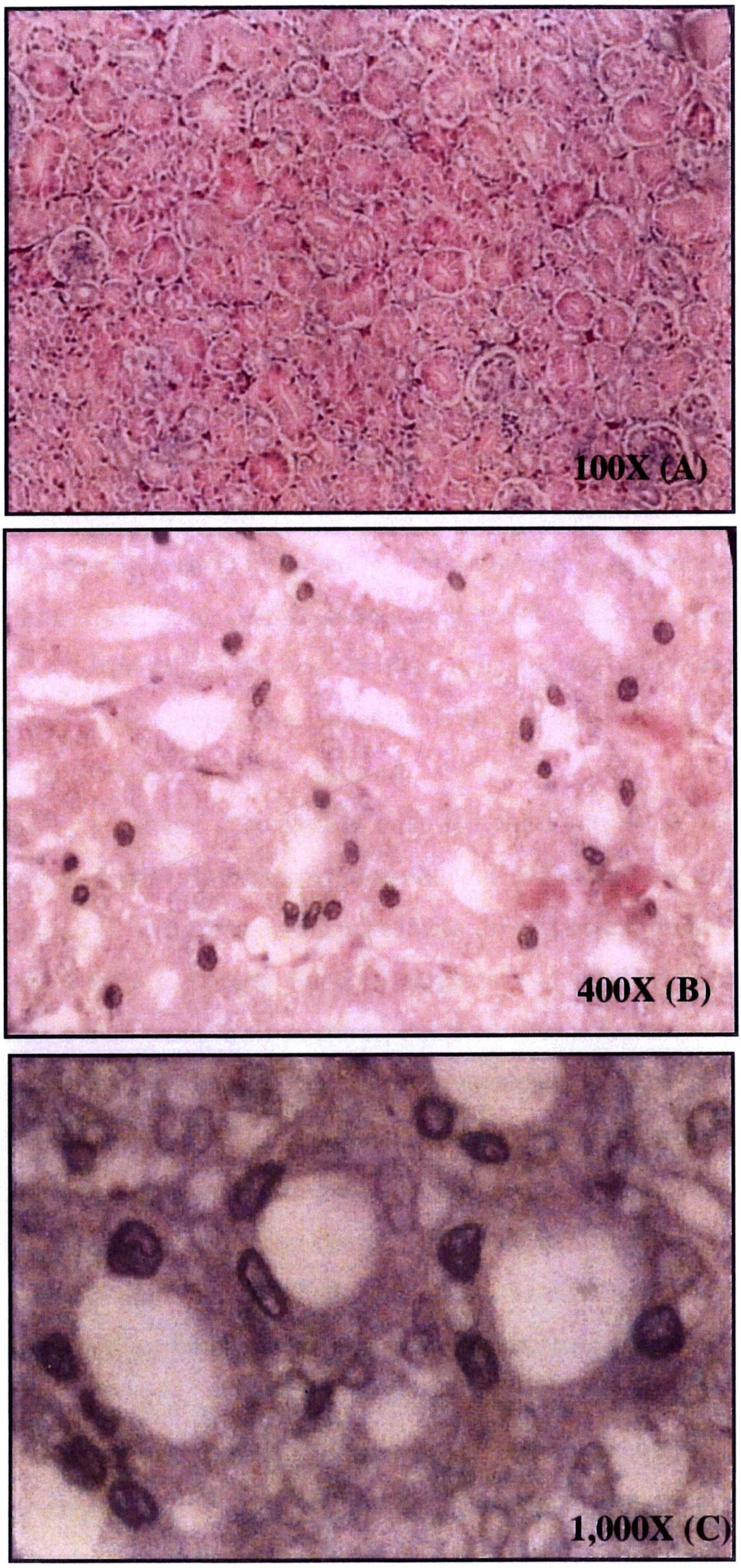

Figure 4.2 A histological view of melamine level 1.00% supplementation in broiler diets for 42 days in the tissues of kidney with magnification 100X(A), 400X(B) and 1,000X (C) under inverted microscopy

4.4 Discussion

The results of this study demonstrated that broilers fed on melamine showed a linear decrease effect on BWG. As the levels of melamine in the diets increased the greatest decrease in BWG was observed in birds fed 1.00% melamine. Feed efficiency or FCR showed similar results as BWG with a less efficient linear FCR as the levels of melamine increased in the diets and also showed the greatest decreased efficiency when fed 1.00% melamine. The results support an early study by Ledoux et al. (2009) who fed graded levels of melamine (0.50-3.00%) to young broilers from hatch to 14 days. No effect was observed in FI among the controls and chickens fed 0.50 or 1.00% melamine, and BWG decreased when fed > 1.00% melamine with the greatest decrease in BWG observed in birds fed > 2.00% melamine. A similar previous study, also reported by Brand et al. (2009) about young turkey poults fed on graded levels of melamine (0.50-3.00%) from hatch to 21 days, indicated that FI reduced in poults fed diets containing 1.50% melamine, whereas BWG reduced in birds fed > 1.00% melamine when compared with the controls.

Survival percentage decreased in birds fed either melamine, UF or their equal mixtures compared with the controls, and showed quadratic effects as the levels of either melamine, UF or their equal mixtures with the greatest decrease occurred when birds were fed 1.00% melamine. Survival percentage showed no difference between birds fed either UF or their equal mixtures, but there was a decrease in survival rate as the levels of melamine increased with the greatest decrease observed when birds were fed on 1.00% melamine. These findings demonstrate that the broilers can tolerate up to 0.50% UF with no adverse effects on survival percentage and that at this level, UF is not toxic to the birds, and when compared with melamine diets, is even less toxic. This study showed that UF can be added at higher levels than melamine. Feeding on melamine decreased survival percentage with the greatest decrease occurring when birds were fed on 1.00% melamine. These results concur with Ledoux et al. (2009) who reported that mortality was observed in chickens fed on 2.50 and 3.00% melamine as early as d-5 of study, and by d-14, mortality was observed at 12, 20 and 30%, respectively, in chickens fed 2.00, 2.50 and 3.00% melamine. Similar results also reported by Brand et al. (2009) in young turkey poults. Significant mortality was observed in turkeys fed 1.50, 2.00 and 3.00% melamine with 27, 63 and 93%

mortality. Furthermore, economic effect was evaluated by the productive index (PI) or the European productive efficiency factor (EPEF) by using BWG, FCR, survival percentage and aging as parameters. This evaluation indicated that melamine, UF or their equal mixtures showed economic lost and quadratic effects as the levels of either melamine, UF or their equal mixtures in the diet increased but no different effects were observed in either carcass dressing percentage or total carcass yield among treatments. However, the liver percentage was higher (P<0.05) than the control. Similar finding have been reported by Gao et.al. (2010). They reported that ducks fed diets supplemented with 100 mg of melamine/kg of feed showed liver cell vaculoation, inflammatory cell infiltration, and fatty degeneration.

The residue levels of melamine in the breast meat and the liver at d-42 were detected when the birds were fed diets containing ≥ 0.50% melamine, adding melamine in the diet alone or their equal mixture of melamine and UF. The distribution of residual melamine in the liver tissues was higher than in the breast meat and after a 7-d withdrawal period, residual melamine was not detected either in the breast meat or the liver. Similar findings have been reported by Lu et al. (2009[a]). They reported that residue levels of melamine in broiler breast, liver and kidney tissues at d28 and 42 were below the detection limit when the diets contained ≤ 50 mg of melamine /kg of diet, and melamine was detected in breast meat and liver only in chicken fed diets containing between 500 and 1000 mg of melamine/kg per diet. The distribution of melamine varied in different tissues, with the lowest concentration in the breast meat and the highest in the kidney. Melamine residue levels in the tissues were lower on d-42 compared with d-28 suggesting that broiler chicks might developed more capability to clear melamine from body tissues with advanced age. Finally, after a 7-d withdrawal period, melamine was not detected in any of the broiler tissues, indicating that broilers had the ability to quickly deplete the tissues of melamine. The results of this study indicate that melamine concentrations of 1.00% in broiler diets did not induce detectable levels of melamine residues either in the breast or liver tissues. This data showed that melamine residues in the breast meat and liver were depleted after the 7-d withdrawal period.

Microscopic examination of crystals with "spoke wheel" appearance in the tissues of the liver, kidney and spleen of broilers fed on diets containing 0.50% melamine revealed either microscopic crystals in various sizes or large white ones that were visible to the naked eye. Similar findings were reported by Brand et al. (2009). They observed that turkey poults fed on a 2.00-3.00% melamine diet contained crystals that were also either microscopic and various in size or large white visible to the naked eye. Lam et al. (2009) and Ledoux et al. (2009) also reported crystal formation in bile and histopathologic lesions in kidneys which revealed crystals similar to those observed in renal tubules of cats with melamine associated renal failure. Furthermore, Bai et al. (2010) examined kidney samples from laying hens administered with melamine at 8.60-140.90 mg/kg of BW/d for 34 days. The crystals were found in one of three kidneys belonging to hens treated with melamine at either 62.60 or 140.90 mg/kg. Reimschuessel et al. (2008) described that chickens fed only melamine could develop spherulite crystals containing uric acid, a normal excretion product of chickens. Previous studies also showed that pigs, fish, cats, and rats fed melamine or cyanuric acid in a 1:1 ratio developed renal crystals composing of melamine-cyanurate (Puschner et al., 2007; Dobson et al., 2008; Reimschuessel et al., 2008). The relationship between the melamine concentration in diets fed to chickens and the melamine crystals appearance, amount and sizes in the liver, kidney and spleen of broilers from this present study was best described by a series of linear functions. Therefore, the results of microscopic examination of the amount and various sizes of golden-brown crystals in the liver, kidney and spleen tissues of broiler chicks are useful for predicting the dietary melamine dosage. Histological of liver tissues showed hepatocyte swelling, congestion, cell membrane less prominent, red blood cell distension and sinusoidal dilatation. Spleen tissues showed congestion, white blood cell infiltration. Melamine crystals were not found in the livers and spleens of any broilers. Kidney tissues showed brown melamine crystals deposited in the interstitial tissue surrounding the renal tubules of laying hens fed 1.00% melamine contaminated in diet. The similar results were also reported by Gao et al. (2010) who supplemented 0 to 100 mg of melamine/kg of duck diet. Their results showed that histological lesions were observed in the kidneys of ducks subjected to $\geq$25 mg/kg melamine. Histological lesions in liver tissues from birds fed melamine supplemented diets were less prominent than those observed in kidneys. Fatty degeneration and liver fibrosis were occasionally observed in the livers of ducks fed diets containing 25 mg of melamine/kg of feed. Piecemeal necrosis and inflammatory cells were present within the adjacent interstitium in some livers of the ducks fed 50 mg of melamine/kg of feed. Liver cell vaculoation, inflammatory cell infiltration, and fatty degeneration were found in some liver of the ducks fed with 100 mg of melamine/kg of feed. Bai et al. (2010) reported that melamine supplement at 17.4 to140.9 mg/kg of body weight (BW) for 34 days, revealed dilated renal tubules and small blood vessel expansion in hens. Furthermore, crystals were found in one of three kidneys of the melamine treated hens levels 62.6 and 140.9 mg/kg of BW per day. Addition, Reimschuessel et al. (2008) described that chickens fed melamine could develop spherulite crystals containing uric acid, a normal excretion product of chickens. Furthermore, previous studies have shown that pigs, fish, cats and rats fed melamine and cyanuric acid in a 1:1 ratio develop renal crystals composed of melamine-cyanurate (Puschner et al., 2007; Dobson et al., 2008; Reimschuessel et al., 2008). However, no change in renal functions was observed in cats and fish treated with either melamine or cyanuric acid alone at doses of 181 or 400 mg/kg of BW (Reimschuessel et al., 2008).

They could also be used as a starting point for estimation of an appropriate tissues withdrawal interval after an accidental exposure to melamine concentration feed under a number of various exposure scenarios. Further research is needed to explore and validate these specific interactions and correct the systematic bias. These techniques can serve as the fundamental for future research to refine and expand the applications to other species.

4.5 Conclusion

The results of this study indicated that over a 42 day feeding period, melamine concentration $\geq$ 0.75% depressed both growth performance, and survival percentage, and also decreased PI. Residue levels of melamine in breast meat and the liver were depleted after a 7-d withdrawal period. On the basis of the above results, assuming that most of the melamine within the animal body existed in a free form, which allowed for rapid depletion at the beginning of the withdrawal period. Higher concentrations of melamine may be widely distributed in different tissues, requiring more time to be cleared. This study provided some information about melamine residues in the breast meat and liver tissues and depletion or elimination time in the breast meat and liver of broilers, as well as demonstrated the risks to human health posed by melamine adulterants used to increase the nitrogen content in feed.