Original Article

Effects of Dietary Melamine or Urea-formaldehyde or Their Mixtures on Performance, Carcass Quality, Melamine Residues and Microscopic Changes in Broiler Tissues

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Abstract

A study was conducted to determine the effects of dietary graded levels of melamine, urea-formaldehyde (UF) and their mixture on growth performance, carcass quality, melamine residues and microscopic changes in broiler tissues from d-1 to d-42 followed by a 7-d feeding of withdrawal diets. One thousand and forty 1-d-old equal mixed-sex Arber Acres broiler were assigned to 13 dietary treatments with 4 replicate pens of 20 chicks assigned to each treatment. The diets of melamine, UF and their mixture contained four graded levels (0.25, 0.50, 0.75 and 1.00%) but no melamine or UF was added to the control diet. There was no difference (p>0.05) in feed intake (FI) among controls and chickens fed on the three products (melamine or UF or their mixture). Body weight gain (BWG) decreased significantly (p<0.05) in birds fed $\geq$ 0.75% melamine, with the greatest decrease in BWG observed in birds fed 1.00% melamine. There was a difference (p<0.05) in BWG between the basal diet and chickens fed on the three products. BWG decreased linearly (p<0.05) with increasing levels of dietary melamine. Chickens that are fed on the three products were less efficient (p<0.05) in converting feed to gain compared with the controls. Survival percentage decreased (p<0.05) in chickens fed all four levels of melamine or fed $\geq$ 0.50% UF or fed 1.00% or a mixture of both compared with the controls. Birds fed with melamine showed a decreased (p<0.05) survival percentage compared with chicks fed UF or a mixture of both. These findings demonstrate that UF has a higher toleration level than melamine. Chickens Fed the four levels of melamine showed a lower (p<0.05) European production efficiency factor (EPEF) compared with birds fed UF, the mixture or the controls. The influence of melamine, UF or their mixture showed both linear (p<0.05) and quadratic (p<0.05) economic lost effects as the level of either melamine, UF or their mixture in the diet increased. There was no difference in carcass dressing percentage and total carcass yield among treatments. Residue levels of melamine in meat and liver tissues were below the detection limit when the diet contained less than 0.50% melamine supplementation in the diets. Tissue melamine levels increased (p<0.05) in both meat and liver tissues with the increasing levels of melamine in the diets. The melamine residues in the liver were higher (p<0.05) than in the breast tissues. A withdrawal period of 7-d was found to clear the tissues of melamine, which means that chickens had an ability to quickly deplete the melamine that accumulated in their tissues. Microscopic examination of the melamine crystal on liver, kidney and spleen tissues provided evidence of a strong correlation between the amount and size of golden brown crystals and diets containing melamine concentration which are useful in the prediction of dietary melamine dosage in the diets.

Keywords: Broiler, melamine, microscopic changes, performance, tissue residue, urea-formaldehyde

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บทคัดย่อ

ผลของการเสริมเมลามีนหรือยูเรียฟอร์มาลดีไฮด์หรือส่วนผสมของทั้งสองชนิดต่อสมรรถนะในการผลิต คุณภาพซาก ปริมาณตกค้างและการเปลี่ยนแปลงทางกล้องจุลทรรศน์ในเนื้อเยื่อของไก่เนื้อ

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การทดลองครั้งนี้เพื่อศึกษาผลการเสริมเมลามีน ยูเรียฟอร์มาลดีไฮด์ (UF) และส่วนผสมของทั้งสองชนิดในอาหารไก่เนื้อต่อสมรรถนะในการผลิต คุณภาพซาก พิษตกค้างและการเปลี่ยนแปลงทางกล้องจุลทรรศนในเนื้อเยื่อของไก่เนื้อตลอดช่วงอายุการเลี้ยง 1-42 วัน ใช้ลูกไก่เนื้ออาร์เบอร์เอเคอร์คละเพศ 1,040 ตัว แบ่งเป็น 13 กลุ่มๆละ 4 ซ้ำ แต่ละซ้ำมีลูกไก่เนื้อ 20 ตัว สูตรอาหารประกอบด้วยการเสริมเมลามีน หรือ UF หรือส่วนผสมของทั้งสองชนิด 4 ระดับ (0.25, 0.50, 0.75 และ 1.00%) และกลุ่มควบคุมไม่มีการให้สารเสริม ผลการทดลองพบว่า อาหารที่กิน (FI) ในทุกกลุ่มทดสอบไม่มีผลแตกต่างกัน (p<0.05) ทางสถิติ การเสริมเมลามีนในอาหาร ≥ 0.75% มีผลทำให้น้ำหนักตัวเพิ่มขึ้น (BWG) ลดลง (p<0.05) และลดลงมากที่สุดเมื่อไก่เนื้อเสริมด้วยเมลามีน1.00% ในสูตรอาหาร การเสริมผลิตภัณฑ์ทั้ง 3 ชนิด เมื่อเปรียบเทียบกับกลุ่มควบคุม พบว่า BWG มีผลแตกต่างกันทางสถิติ (p<0.05) BWG จะลดลงเป็นเส้นตรง (p<0.05) เมื่อระดับเมลามีน ในสูตรอาหารเพิ่มขึ้น ไก่เนื้อที่เลี้ยงด้วยอาหารทั้ง 3 ชนิด จะมีประสิทธิภาพการเปลี่ยนอาหารให้เป็นเนื้อได้ต่ำกว่า (p<0.05) ไก่เนื้อที่เลี้ยงด้วยสูตรควบคุม อัตราการเลี้ยงรอดลดลง (p<0.05) เมื่อเสริมด้วยเมลามีน ทั้ง 4 ระดับ หรือเลี้ยงด้วยอาหารที่มี UF ≥ 0.50% หรือส่วนผสมของทั้งสอง 1.00% เมื่อเปรียบเทียบกับไก่เนื้อที่เลี้ยงด้วยสูตรควบคุม ไก่เนื้อที่เลี้ยงด้วยสูตรอาหารเสริมเมลามีน จะให้อัตราการเลี้ยงรอดต่ำกว่า (p<0.05) ไก่เนื้อที่เลี้ยงด้วยสูตรอาหารที่เสริม UF หรือส่วนผสมของทั้งสองชนิด ผลการทดลองครั้งนี้แสดงให้เห็นว่าไก่เนื้อสามารถทนต่อสาร UF ได้ในระดับสูงกว่า เมลามีน การเสริมเมลามีนในอาหารทั้ง 4 ระดับ แสดงผลตอบแทนทางเศรษฐกิจ (European production efficiency factor, EPEF) ต่ำกว่า (p<0.05) ไก่เนื้อที่เสริมด้วย UF หรือส่วนผสมของทั้งสองชนิดหรือสูตรควบคุม การเสริมผลิตภัณฑ์ทั้ง 3 ชนิด จะแสดงผล EPEF ลดลงเป็นทั้งเส้นตรง (linear, p<0.05) และเส้นโค้ง (quadratic, p<0.05) ต่อระดับการเสริมของแต่ละผลิตภัณฑ์ที่สูงขึ้นในสูตรอาหาร คุณภาพของซากไก่เนื้อเมื่อสิ้นสุดการทดลองเมื่อวัดเป็นเปอร์เซ็นต์ซากตกแต่ง (Dressing percentage) และ ปริมาณเนื้อทั้งหมด (Total carcass yield) พบว่า ให้ผลไม่แตกต่างกันต่อทุกกลุ่มที่ทดสอบ การตกค้างของ เมลามีน ในเนื้ออกและตับไม่สามารถตรวจวัดได้เมื่อไก่เนื้อกินอาหารที่เสริมเมลามีน ต่ำกว่า 0.50% การตกค้างของเมลามีน ในเนื้อเยื่อของเนื้ออกและตับจะเพิ่มขึ้นเมื่อระดับของการเสริมเมลามีน ในอาหารเพิ่มขึ้น เนื้อเยื่อตับจะมีเมลามีน ตกค้างสูงกว่า (p<0.05) เนื้อเยื่อของเนื้ออก การตกค้างของเมลามีน ในเนื้อเยื่อของเนื้ออกและตับจะหมดหรือไม่พบเมื่อไม่เสริม เมลามีน ในอาหารเป็นเวลา 7 วัน ผลการทดลองครั้งนี้แสดงให้เห็นว่าไก่สามารถขับเมลามีนที่สะสมในกล้ามเนื้อออกจากร่างกายได้รวดเร็ว ผลการตรวจสอบเมลามีนคริสตอล ในเนื้อเยื่อของตับ ไต และม้าม แสดงผลอย่างเด่นชัดต่อสหสัมพันธ์ ระหว่างปริมาณและขนาดคริสตอลสีน้ำตาลทองที่พบในเนื้อเยื่อต่อระดับของเมลามีน ที่เสริมเพิ่มขึ้นในสูตรอาหาร ซึ่งจะเป็นประโยชน์ในการนำข้อมูลนี้ไปทำนายการเสริมเมลามีนที่ปนเปื้อนในสูตรอาหารได้

คำสำคัญ: ไก่เนื้อ เมลามีน สมรรถนะการผลิต การเปลี่ยนแปลงทางกล้องจุลทรรศน์ การตกค้างในเนื้อเยื่อ ยูเรียฟอร์มาลดีไฮด์

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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 satisfying 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 (Burns, 2007[a]; Thomas and Kulkarni, 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[b]; 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 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 aqua feeds. Chicken diets are often pelleted to improve gains or feed utilization (Lanson and Smyth, 1955; Proudfoot and Hulan, 1980). Stilborn et al. (1991) were 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 hens were 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 effects were seen on performance and also no adverse effects were noted on blood parameters. The results of these studies indicated that urea-formaldehyde resin pellet binder could 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 >10 g/d caused 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 and 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 to young broilers from hatch to 14 days and indicated that feed intake 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 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. (2009[a]) fed graded levels of melamine (5-1000 mg melamine/kg of diets) to broilers throughout a 42-d periodand cocluded that melamine had 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 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. (2009[b]) reported that Cherry Valley ducks fed < 1000 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) to laying ducts for 21 days followed by a 21 day withdrawal period and 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 rats fed melamine or cyanuric acid in a 1:1 ratio develop renal crystals composing 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.

Materials and Methods

Melamine (M) 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 were diluted with ground corn to a concentration of 10%. Then, appropriate amounts of these diluted materials were then mixed in basal diets (Table 1) to prepare treatment diets with graded levels of melamine, UF or equal mixture of melamine and UF.

Table 1 Composition of the basal diets (as-fed basis).

Item	Starter 1 1-14 d	Starter 2 15-28 d	Grower 29-35 d	Finisher 36-49 d
Ingredient (%)				
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 D3: 3,528 IU, vitamin E: 33 IU, K3: 0.91 mg, thiamine: 2 mg, vitamin B1: 18 mg, vitamin B2: 8 mg, nicotinic acid: 55 mg, pantothenic acid: 18 mg, vitamin B6: 5 mg, vitamin B12: 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.

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 the 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 equal mixture of both 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 1). The diets were fed in mash form. Feed providing *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 illness or behavioral changes, and mortality was recorded daily.

Measurements and analysis: Chickens' 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). On d 28, and 42, one chick was randomly selected from each replicate pen and euthanized by cervical dislocation, samples of the breast meat, liver, kidney and spleen were collected and kept at -20^{o}C until examination conducted by using an inverted microscope fitted with a digital video camera. 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 was recorded and all of the data was expressed as percentage of the pre-slaughter weight of the same bird. Tissue samples (5 g) were extracted according to Lu et al. (2009[a]). The melamine concentrations were determined using HPLC method as described 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 (Stillborn et al., 1991), therefore the UF concentration were not determined in these studies.

Results

Growth performance: Table 2 describes the effects of dietary melamine, UF and their mixture on growth performance. There was no difference (p>0.05) in FI among controls and chickens fed all graded levels of melamine, UF or their mixture. BWG decreased significantly (p<0.05) in birds fed $\geq$ 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 the mixture compared with controls. There were significant linear (p<0.05) effects on BWG as the levels of melamine in the diets increased. There was no difference in FI among the controls and chickens fed 0.50 or 1.00% melamine. Feed conversion ratio (FCR) was significantly less efficient (p<0.05) for birds fed either melamine, UF or their mixture 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 the mixture.

Survival percentage, European productive efficiency factor and carcass yield percentage: The survival percentage significantly decreased (p<0.05) in birds fed either melamine, UF or their mixture compared with the controls (Table 2). Survival percentage did not differ (p>0.05) between birds fed diets containing UF or the mixture. There was a significant quadratic (p<0.05) effect on the survival percentage as the levels of either melamine, UF or their mixture in the diets increased. The survival percentage did not differ (p>0.05) between birds fed either UF or an equal mixture of both melamine and UF, but showed a greatest decrease when birds were fed on 1.00% melamine. Survival percentage also decreased (p<0.05) in chickens fed 1.00% UF and also 1.00% of an equal mixture of both melamine and UF, but there was no difference (p>0.05) in BWG, FI and FCR compared with the controls. The European productive efficiency factor (EPEF) significantly decreased (p<0.05) in birds fed either melamine or UF or their mixture compared with the controls. The influence of melamine, UF or their mixture of the EPEF showed economic lost significant linear (p<0.05) and quadratic (p<0.05) effects as the levels of either melamine, UF or their mixture of the diets increased. There was no difference (p>0.05) in the means of carcass dressing percentage and total carcass yield among treatments (Table 2) throughout the entire experiment. Dietary melamine, UF and their mixture tended to decrease in both dressing percentage and total carcass yield.

Table 2 Effect of dietary melamine (M) or urea-formaldehyde (UF) or equal mixture of both on broiler performance, European productive efficiency factor (EPEF) and carcass efficiency of broiler chicks measured at 42-d of age (% of live body weight).

Treatment		BWG (g)	FI (g)	FCR (g feed/ g BWG)	Survival (%)	EPEF*	Dressing percentage	Total carcass yield (%)
Product	Concentration (%)							
Basal	0	1967^{a}	3740	1.907^{b}	95.83^{a}	236.86^{a}	81.47	51.86
M	0.25	1736^{ab}	3701	2.136^{ab}	90.63^{b}	176.36^{bc}	81.08	50.85
M	0.50	1739^{ab}	3735	2.160^{ab}	89.59^{bc}	173.81^{bc}	80.01	51.70
M	0.75	1745^{ab}	3785	2.168^{ab}	85.42^{c}	164.68^{bc}	80.07	49.21
M	1.00	1611^{b}	3620	2.263^{a}	83.34^{d}	144.22^{c}	80.59	50.54
UF	0.25	1786^{ab}	3672	2.055^{ab}	93.75^{ab}	195.95^{abc}	81.73	50.74
UF	0.50	1769^{ab}	3855	2.179^{ab}	92.71^{ab}	180.00^{b}	81.24	49.64
UF	0.75	1668^{ab}	3619	2.170^{ab}	91.67^{ab}	168.46^{bc}	81.07	50.75
UF	1.00	1693^{ab}	3704	2.188^{ab}	90.63^{b}	168.96^{bc}	81.73	51.13
M+UF	0.25	1807^{ab}	3607	1.997^{ab}	92.71^{ab}	199.87^{ab}	81.40	51.38
M+UF	0.50	1899^{ab}	3753	1.976^{ab}	91.67^{ab}	210.09^{ab}	81.91	50.69
M+UF	0.75	1839^{ab}	3889	2.115^{ab}	90.63^{b}	189.36^{abc}	82.03	50.64
M+UF	1.00	1906^{ab}	3745	1.965^{ab}	89.59^{bc}	207.65^{ab}	81.00	50.18
Pooled SEM		0.090	0.125	0.093	1.405	8.2115	0.871	0.820
		--Probability--						
Product (P)		*	NS	**	**	*	NS	NS
Concentration (C)		NS	NS	NS	**	NS	NS	NS
P x C		NS	NS	*	NS	NS	NS	NS
Contrast								
Control vs other		**	NS	**	*	*	NS	NS
L^1		**	NS	*	NS	NS	NS	NS
Q^1		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

$^{a-d}$Means within a column with no common superscript differ significant (p<0.05).
*EPEF: [survival (%) x BWG (kg)/age(d) x FCR] x 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.

Residual melamine concentration in tissues: Residual melamine concentration in breast meat and liver on d-42 and d-49 are summarized in Table 3. The residue levels of melamine in the breast meat and liver on d-42 were below the detection limit when the diets contained $\leq$ 0.50% melamine supplementation. At d-42, melamine residue 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 mixture of melamine and UF (50:50/w:w), but melamine was detected in the breast meat and liver only in birds fed a diet containing the equal mixture of both 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 7-d withdrawal period, residual melamine was not detected in both breast meat and liver.

Table 3 Concentration of melamine (M) residues in the 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		

$^{a\text{-}f}$ 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$\pm$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.

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 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 on 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" on the liver, kidney and spleen of the chickens fed on graded levels of melamine (0, 0.25, 0.50, 0.75 and 1.00% melamine in the diets) are presented in Figure 1.

Discussion

The results of this study demonstrated that chickens 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 agree with 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 mixture compared with the controls and showed quadratic effects as the levels of either melamine, UF or their mixture with the greatest decrease occurred when birds were fed on 1.00% melamine. Survival percentage showed no difference between birds fed either UF or equal mixture, 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 European productive efficiency factor (EPEF) or the productive index (PI) by using BWG, FCR, survival percentage and aging as parameters. This evaluation indicated that melamine, UF or an equal mixture of both showed economic lost and quadratic effects as the levels of either melamine, UF or their mixture in the diet increased but no different effects were observed in either carcass dressing percentage or total carcass yield among treatments.

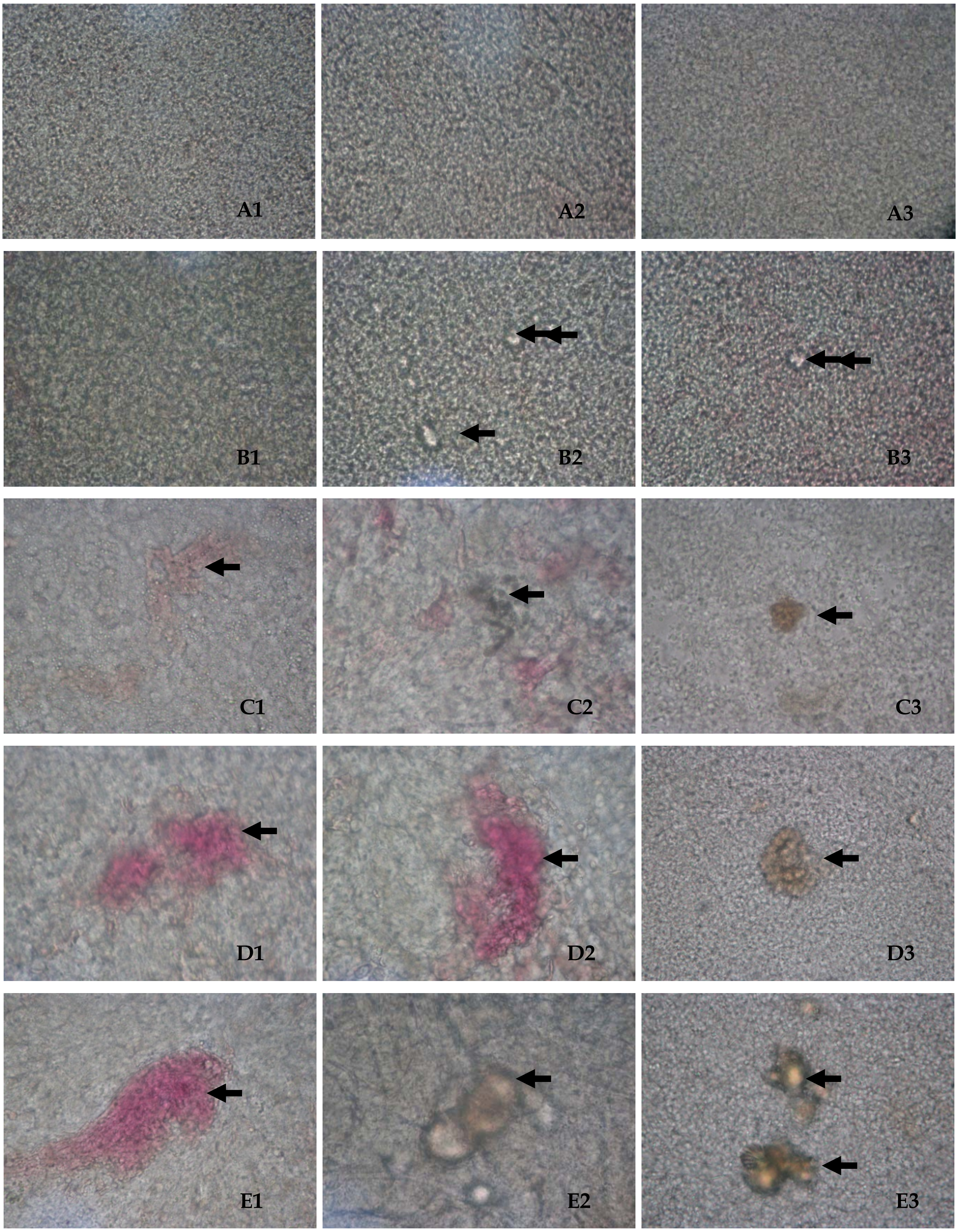

Figure 1. A microscopic view of the graded levels of dietary melamine supplementation in broiler diets for 42 days in the tissues of the liver (A1-E1), kidney (A2-E2) and spleen (A3-E3) with magnification 400-1000X under inverted microscopy: (A) control; (B) 0.25% melamine in the diet; (C) 0.50% melamine in the diet; (D) 0.75% melamine in the diet; (E) 1.00% melamine in the diet. 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 dosage (Figure B1-B3, C1-C3, D1-D3, E1-E3).

The residue levels of melamine in the breast meat and the liver at d-42 were detected when the birds were fed diets containing $\geq$ 0.50% melamine, adding melamine in the diet alone or an 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 $\leq$ 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. 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 basis for future research to refine and expand the applications to other species.

The results of this study indicated that over a 42-d feeding period, melamine concentration of $\geq$ 0.75% depressed both growth performance, and survival percentage, and also decreased EPEF. Residue levels of melamine in the breast meat and the liver were depleted after a 7-d withdrawal period. On the basis of the results above, most of the melamine within the animal body existed in a free form, which allowed a 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 of 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.

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