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Residues of melamine and cyanuric acid in milk and tissues of dairy cows fed different doses of melamine

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ABSTRACT

Melamine (1,3,5-triazine-2,4,6-triamine) may be degraded into cyanuric acid and some other compounds by rumen microorganisms. This study was conducted to assess the transfer of melamine and cyanuric acid in to milk and tissues by dairy cows fed different doses of melamine. Forty mid-lactation dairy cows (157 ± 43 d in milk, 20.8 ± 1.4 kg of milk/d) were divided into 4 groups (n = 10/group) using a completely randomized design. The groups were fed the following amounts of melamine (purity ≥99.5%) at 0 (control), 300 [treatment (Trt) 1], 500 (Trt 2), and 1,000 (Trt 3) mg/d per cow, respectively. The trial lasted for 18 d (12-d feeding period, followed by a 6-d clearance period). Milk samples were collected from the 4 groups on d 1, 2, 3, 4, 8, 12, 13, 14, 15, and 18, and analyzed for melamine and cyanuric acid. On d 13, 3 cows from Trt 2 and Trt 3 were randomly selected and slaughtered; tissue samples including kidney, liver, mammary, bladder, gluteus medius, and longissimus dorsi were collected for melamine and cyanuric acid analyses. Milk and tissue samples were analyzed for melamine and cyanuric acid using a simultaneous liquid chromatography tandem mass spectrometry procedure. Neither melamine nor cyanuric acid was detected in concentrated feed that was being fed to the cows. In melamine-treated groups, milk melamine concentration increased quickly and reached a stable level by d 4 and was at similar levels on d 8 and 12 after the first administration of melamine. Milk melamine levels in treated groups were 0.18, 0.27, and 0.50 mg/L for Trt 1, Trt 2, and Trt 3, respectively, and were highly correlated (R^2 = 0.91) with melamine dosing levels. No cyanuric acid was detected in any of the milk collected from the various groups. Melamine residue levels in tissues of Trt 3 were about 2-fold higher than that in Trt 2, with the highest concentration being found in kidney. No differences in cyanuric acid levels in tissues were found between Trt 3 and Trt 2. Liver, kidney, and bladder tissues were found to contain the highest cyanuric acid levels. This study shows a relationship between dietary melamine levels and cyanuric acid levels found in tissues, which might be the result of melamine being converted to cyanuric acid by microorganisms in the rumen.
Key words: melamine, cyanuric acid, dairy cow, milk and tissue

INTRODUCTION

Melamine (1,3,5-triazine-2,4,6-triamine) is a relatively stable substance that is mainly used for the production of melamine resins and plastics (Subrayan and Rasmussen, 1995; Weil and Choudhary, 1995). Although melamine is believed to have low oral toxicity because of its stable and insoluble properties that prevent it from being hydrolyzed in the body (Newton and Utley, 1978), excessive intake by human or animals has been shown to cause crystalluria and renal damage (Brown et al., 2007; Shen et al., 2010). The European Food Safety Authority (EFSA, 2008) recommends that melamine total daily intake (**TDI**) be less than 0.5 mg/kg of BW. The US Food and Drug Administration (FDA, 2007) guidelines recommend that TDI of melamine and its analogs should be less than 0.63 mg/kg of BW per day for adults and 0.063 mg/kg of BW per day for infants, respectively (FDA, 2008b). The addition of melamine to animal feeds or human foods is strictly prohibited.

Several studies have shown that melamine is not metabolized by the body and is excreted by the kidneys (Newton and Utley, 1978; Lam et al., 2009). Mast et al. (1983) reported that 90% of ingested melamine would be excreted by kidneys within 24 h. Baynes et al. (2008) demonstrated that body distribution of melamine may be limited to the body water fraction and melamine was unlikely to be bound in significant amounts to the tissues. When large quantities of melamine are ingested that exceed the excretion capability of kidney, then renal disease and even death may occur in animals (Ogasawara et al., 1995; Puschner et al., 2007; Dobson et al., 2008) and humans (Lam et al., 2009). However,

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Jutzi et al. (1982) and Shelton et al. (1997) showed that melamine could be metabolized by *Pseudomonas* spp. and *Klebsiella terragena* to form cyanuric acid in vitro. Other recent studies showed increased toxicity resulting from combined exposure of animals to melamine and cyanuric acid (Puschner et al., 2007; Reimschuessel et al., 2008), which raises a high degree of uncertainty with regard to the assessment of risk and safety in animals consuming melamine. The FDA (2008a) has applied an additional 10-fold safety factor to compensate for these uncertainties and recommends that the TDI of melamine be <0.063 mg/kg in food and food ingredients other than infant formula.

Most previous studies have focused on monogastric animals, but melamine metabolism in dairy cows and residues in milk have not been studied. Given the complex and diverse microbial population that is present in the rumen, we hypothesize that melamine might be transformed by ruminal microbes (bacteria, fungi, and protozoa) to form cyanuric acid and other related compounds, such as ammelide and ammeline. These degradation products may find their way into milk and tissues when dairy cows consume feed tainted with melamine. Therefore, the objective of this study was to investigate the transfer of melamine and cyanuric acid (a rumen metabolite of melamine) into milk and tissues of dairy cows receiving different doses of melamine.

MATERIALS AND METHODS

Experimental Design and Treatments

The study was performed at the Beijing Cangdafu Dairy Farm (Beijing, China). Animal care and procedures were approved and conducted under established standards of the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China. Health of cows was monitored continuously before and during the experimental period.

Forty mid-lactation Holstein dairy cows (157 ± 43 DIM, 20.8 ± 1.4 kg of milk/d, and 2.9 ± 0.8 parity) were randomly assigned to 1 of 4 groups (n = 10/group). The whole trial lasted for 18 d, during which the first 12 d was the feeding period and the last 6 d was the clearance period. In the feeding period, cows were dosed with melamine at 0 (control), 300 [treatment (**Trt) 1**], 500 (**Trt 2**), and 1,000 (**Trt 3**) mg/d per cow, respectively. The melamine (purity ≥99.5%) used in this trial was obtained from Beijing Chemical Reagent Company (Beijing, China). During the first 12 d (feeding period), before each morning feeding, cows were allowed to consume all the added melamine mixed with about 35 g of concentrate (composed of 50% soybean meal and 50% corn powder). During the last 6 d of the 18-d feeding period, the melamine was removed from the diet and the cows were monitored for the 6-d clearance period.

All cows were fed the same basal diet (Table 1) as a TMR, which was formulated to satisfy the nutrient recommendations for 25 kg/d of milk production according to the *Feeding Standards of Dairy Cattle* (China NY/t34, 2004). Ingredients and chemical composition of the basal diet are listed in Table 1. The TMR was fed 3 times daily at 0730, 1430, and 2000 h in amounts to ensure approximately 5% refusals. Cows were milked 3 times daily (0930, 1530, and 2300 h) and individual cow milk yields were recorded for each milking.

Sampling and Analysis

Before the trial started, melamine and cyanuric acid in the samples of feed ingredients and milk from all the cows were analyzed for determination of potential background concentration. During the experimental periods, concentrate and forage ingredient samples were collected on d 1, 5, 10, 15, and 19 of the experimental period and analyzed to determine background concentrations of melamine.

Table 1. Ingredient composition and nutrient levels of the basal diet (DM basis)

Item	% of Ration DM
Ingredient	
Corn silage	28.00
Chinese wild rye hay	14.00
Brewers grain	7.50
Bean curd residue	6.00
Corn	19.00
Cottonseed meal	6.50
Wheat bran	3.70
Soybean meal	1.75
Peanut meal	2.40
Yeast powder	2.75
Scrap tankage	3.10
Corn embryo cake	1.85
Salt	0.50
Premix[1]	0.50
Sodium bicarbonate	0.90
Calcium monohydrogen phosphate	0.40
Limestone	0.75
Calcium carbonate precipitated light	0.40
Chemical analysis[2]	
NE_L[3] (MJ/kg of DM)	6.23
CP	15.92
Ether extract	4.69
NDF	41.30
ADF	22.97
Ca	0.72
P	0.36

[1]Contained (per kilogram of DM) a minimum 250,000 IU of vitamin A; 65,000 IU of vitamin D; 2,100 IU of vitamin E; Fe, 400 mg; Cu, 540 mg; Zn, 2,100 mg; Mn, 560 mg; Se, 15 mg; I, 35 mg; Co, 68 mg.

[2]Analyzed value.

[3]Calculated value (based on China NY/t 34, 2004).