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Molybdenum and Cadmium exposure influences the concentration of trace elements in the digestive organs of Shaoxing duck (*Anas platyrhyncha*)

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ABSTRACT

To investigate the toxic effects of Molybdenum (Mo) and Cadmium (Cd) on trace elements in digestive organs of Shaoxing duck (*Anas platyrhyncha*), 120 Shaoxing ducks were randomly divided into control group and 5 treatment groups which were treated with a commercial diet containing different dosages of Mo and Cd. On the 60th and 120th days, the beak, esophagus, glandular stomach, muscular stomach, small intestine, large intestine and feces were collected to determine contents of Mo, Cd, copper (Cu), iron (Fe), zinc (Zn) and selenium (Se), then correlation analysis was performed. The results showed that Cd content in digestive organs significantly increased in co-treated groups compared to single treated groups and Mo concentration increased in Mo-treated groups compared to control group, whereas Cu, Fe, Zn and Se concentrations in digestive organs decreased in co-treated groups. Furthermore, Cd and Mo were mainly accumulated in the small intestine and esophagus, respectively. There was a strongly positive correlation between Cd and Mo while they had negative correlation with Cu, Fe, Zn and Se, respectively. In feces, Mo and Fe contents in high dose of Mo group and high Mo combined with Cd group were significantly higher than those in control group, and Cu content in all treated groups significantly increased and Cd, Zn and Se concentrations had no difference. The results indicated that dietary Mo or/and Cd might disturb homeostasis of trace elements in digestive organs of Shaoxing duck. Moreover, the two elements presented a synergistic relationship.

1. Introduction

Molybdenum (Mo) is an essential trace element for animals and universally distributed in the environment and organisms (Trumbo et al., 2001). It is used in industrial and agricultural activities that lead to increase of Mo environmental concentration (Kumchai et al., 2013; Raisbeck et al., 2006; Swan et al., 1998). Excessive Mo enters animals and then accumulates in body. High dose of Mo can cause adverse effects on the ovary, testicle, kidney, spleen and bones in aquatic and terrestrial animals (Barceloux, 1999; Bersenyi et al., 2008; Cao et al., 2016a; Liao et al., 2017; Xia et al., 2015; Raisbeck et al., 2006; Swan et al., 1998). In addition, the toxicity of Mo can disrupt the balance of trace elements in the body. Mo has an obvious antagonism to combined zinc (Zn) and copper (Cu), respectively (Aupperle et al., 2001). Molybdenosis usually occurs under natural grazing conditions (animals have free access to drinking water and diet in the wild) in many parts of the world (Mills and Davis, 1987).

Cadmium (Cd) is one of the most important environmental heavy metal toxicants. The major sources of Cd exposure in the environment occur in industries involved with Cd mining and refining, electroplating, battery, plastics, pigment and electronics production. It enters the body of animals and efficiently accumulates with long biological half-life (Cannino et al., 2009; Rani et al., 2014; Shih et al., 2004). Chronic exposure to Cd can cause adverse effects on the kidney, liver, lung, pancreas, testes, placenta, spleen and bones (Angeli et al., 2013; Chen et al., 2017; Erboga et al., 2016; Kundu et al., 2009). In addition, Cd can disturb the homeostasis of trace elements (Martelli et al., 2006; Nemmiche et al., 2011).

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Table 1
Composition and nutrient levels in the basal diet for the ducks and concentration of Mo, Cd, Cu, Fe, Zn and Se in the basal diet and water.

Composition of diet	Content (%)		Nutrient levels	Level		Trace elements	Duckling feed (μg/g)	Duck feed (μg/g)	Water (μg/mL)
Ingredient	0–3 week	After 3 week	Index	0–3 week	After 3 week				
Soybean meal	18.00	20.00	Ca (%)	0.800	2.77	Mo	4.15	4.73	0.010
Corn	59.99	44.00	DE ($MJ{\cdot}kg^{-1}$)	11.93	11.44	Cd	0.247	0.476	0.008
Wheat bran	11.00	14.40	Crude protein (%)	18.03	17.63	Cu	191	109	0.021
Rice bran	—	11.00	Met + Cys (%)	0.600	0.650	Fe	748	709	0.188
Cottonseed meal	5.00	—	Total phosphorous (%)	0.670	0.700	Zn	211	190	0.152
Bone meal	1.58	5.80	Lys (%)	0.850	0.970	Se	1.05	1.05	0.015
Fish meal	3.00	2.00	Available phosphorus (%)	0.350	0.400				
Salt	0.370	0.300							
Met	0.060	0.100							
$CaHPO_4$	—	1.40							
Premix*	1.00	1.00							
Total	100	100							

* *Per kilogram of premix contained the followings:* VD_3 400 IU, VA 2500 IU, VB 1215 μg, VK_3 0.500 mg, VE 10.00 mg, Riboflavin 4.00 mg, Thiamine 4.00 mg, Nicotinic acid 55.00 mg, Pantothenic acid 11.00 mg, Biotin 0.080 mg, Pyridoxine 2.50 mg, Folic acid 1.00 mg, Choline 1300 mg, Se 0.200 mg, Fe 80.00 mg, Cu 10.00 mg, Zn 60.00 mg, Mn 50.00 mg.

Due to their extensively industrial and agricultural utilization, Mo and Cd environmental concentrations are rapidly increasing. In China, it is rich in tungsten ore. In the mining and screening processes of tungsten ore, Mo combined with Cd in the tailing usually pollute environment and waterfowl. Our study showed Mo and Cd presented a synergistic effects on the damage of kidney (Xia et al., 2015), but the effect of Mo combined with Cd on trace elements in digestive organs of duck is not well understood. Many studies have been carried out using Shaoxing duck (Wu et al., 2012a, 2012b, 2014; Zhang et al., 2012). Because Shaoxing duck is the main breed in Southern China, which is also the main breed in Southern Jiangxi province polluted by Mo and Cd. The duck has many advantages such as high laying rate, long egg production peak duration, high feed utilization rate, and strong life force. It is one of the most popular breeds in China. Thus we also used this species in the experiment. Shaoxing duck models of sub-chronic poisoning with Mo or/and Cd were established to not only observe a clear effect of Mo or/and Cd on contents of Mo, Cd, Cu, Zn, iron (Fe), selenium (Se) in duck digestive organs and feces but also investigate the relationship of Mo and Cd and the relationship of Mo, Cd and Cu, Fe, Zn and Se, thus providing evidence to elucidate the toxicity of Mo or/and Cd on duck.

2. Materials and methods

2.1. Animals and treatments

All animals care and experimental procedures were approved by the institutional ethics committee of Jiangxi Agricultural University. A total of 120 healthy 1-day-old Shaoxing ducks were obtained from Zhuji Guowei poultry Co., Ltd., Zhejiang Province and kept in stainless steel positive/negative pressure isolator (CCJH-1, Tianjin Jinhang purification air conditioning Engineering Co., Ltd., China). After they were advanced reared for 10 d without abnormality by strict clinical examination, the trail was began. Shaoxing duck models of excessive exposure to Mo or/and Cd were developed as described in our previous publication (Cao et al., 2016b). Briefly, 120 healthy 11-day-old Shaoxing ducks were randomly divided into 6 groups (n = 20 per group). Shaoxing ducks in each group were fed with basal diet with different concentrations of Mo or/and Cd: control group (0 mg/kg Mo, 0 mg/kg Cd), low-dose Mo diet group (LMo group, 15 mg/kg Mo), high-dose Mo diet group (HMo group, 100 mg/kg Mo), Cd group (4 mg/kg Cd), LMoCd group (15 mg/kg Mo, 4 mg/kg Cd), and HMoCd group (100 mg/kg Mo, 4 mg/kg Cd). The basal diet was formulated according to the National Research Council (NRC) (1994). The trace elements content of the basal diet in control group were based on nutrient requirements for poultry (NRC). And Cd content in the basal diet were below the normal allowable amount of feed standard (≤ 0.5 mg/kg). Treated groups' diet were added Mo or/and Cd on the basis of the control group's diet, respectively. Hexaammonium molybdate ($(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$) and cadmium sulfate ($3CdSO_4{\cdot}8H_2O$) were used for Mo and Cd sources in this experiment, respectively. There are differences in nutritional needs of animals at various growth stages, thus Shaoxing ducklings were fed with duckling basal diet and Shaoxing duck basal diet before and after 21-day-old, respectively. The toxic study of heavy metals to poultry, different diets was also used in other study according to the different growth stages of poultry (Karimi et al., 2013). The feeding experiment lasted for 120 days, and Shaoxing ducks were given free access to diet and water. The animals were handled and treated in accordance with the strict guiding principles of the National Institution of Health for experimental care and use of animals. The composition of basal diet for ducks and the concentration of Mo, Cd, Cu, Fe, Zn and Se in the basal diet and water are shown in Table 1.

2.2. Sample collection

On the 60th day and 120th day, 10 Shaoxing ducks from each group were randomly selected after fasting for 12 h, they were sacrificed with an overdose intravenous injection of sodium pentobarbital (Nembutal, Abbot Labs, IL, USA, 50 mg/kg), the beak, esophagus, glandular stomach, muscular stomach, small intestine and large intestine were immediately removed. After that, all samples were washed with deionized water and stored at −20 °C for determining contents of Mo, Cd, Cu, Fe, Zn and Se. Feces were collected before fasting on the 60th day and 120th day and stored at −20 °C for determining trace elements.

2.3. Preparation of sample digestion for atomic absorption

The collected water samples were wet-digested with 10% HNO_3. Each duck's entire beak, esophagus, glandular stomach, muscle stomach, small intestine and large intestine were dried at 80 °C for 24 h in drying oven. Then samples were grinded by high flux lapping instrument (Tissuelyser-24, Shanghai Jingxin Industrial Development Co., Ltd., China). About 5.0 g of each grinded sample baked at 110 °C in drying oven until the sample was constant weight. The water content (%) for the beak, esophagus, glandular stomach, muscle stomach, small intestine and large intestine were 46.82 ± 0.21%, 70.16 ± 0.28%, 66.69 ± 0.37%, 66.2 ± 0.70%, 66.17 ± 0.87%, 66.56 ± 0.32% on 60th day, respectively, they were 37.18 ± 0.12%, 67.37 ± 0.35%, 64.7 ± 0.49%, 65.39 ± 0.61%, 64.24 ± 0.87%, 64.96 ± 0.17% on 120th day, respectively. Then, about 1.0 g of each sample was