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Study on Zn relative concentration and chemical state in broilers duodenum by micro-X-ray fluorescence and micro-X-ray absorption fine structure

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ABSTRACT

Micro-X-ray fluorescence (μ-XRF) and micro-X-ray absorption fine structure (μ-XAFS) are not widely used in animal science. The objective of this experiment was to employ μ-XRF and μ-XAFS technique to determine the change of Zn quantity, distribution and chemical state (Zn^{2+} chemical state, organic zinc chemical state) into intestinal wall using different zinc sources. 45 newly hatched healthy (1-d-old) commercial male broilers were used in this experiment. The chicks were fed a corn–soybean meal basal diet (90.50 mg/kg) from day 1 to 21, but were fed a semipurified diet (12.51 mg/kg) after day 21 to deplete the body Zn stores. At 28 d of age, after an overnight fast, 45 birds were randomly allotted to 3 perfusion groups (ZnMet, ZnLys, $ZnSO_4$) with 15 replicates/group for in situ ligated intestinal loops of broilers in experiment. Duodenum of each bird was used as 1 replication of intestinal segments. The solutions injected into the duodenal loops were buffered with 15.5 mmol/L of morpholineoethanesulfonic acid. In the treatment groups of different Zn sources, 0.616 mmol/L (40 mg/L) was added to the media. The 3.5 mL of Zn dose was injected and incubated 30 min in the abdomen cavity. The μ-XRF and μ-XAFS were used to analyze the relative quantity, distribution and chemical state of Zn in the intestinal wall, and atomic absorption spectrometry (AAS) was used to verify Zn concentration in the whole intestinal sac. The results showed that ZnMet and ZnLys group samples have a greater amount of zinc in the intestinal wall specific region than $ZnSO_4$ group. The Zn chemical state of organic Zn and $ZnSO_4$ group were identical in intestinal wall specific region. In addition, the Zn concentration achieved using ZnMet was greater than that for ZnLys and $ZnSO_4$ ($P < 0.05$) as measured by AAS. As observed in this experiment, organic zinc was more easily absorbed than inorganic Zn.

1. Introduction

Zinc plays an essential role in a wide variety of biochemical processes and is a required cofactor for the function of over 300 different enzymes (Vallee and Auld, 1990; Gaither and Eide, 2001). It is known as a "life element" with a wide range of physiological and biochemical functions in vivo. Zinc is used in animal feed in both the inorganic and organic states. Some feeding studies demonstrated that increased bioavailability in organic Zn sources compared with inorganic sources in rats (Dong, 2001), pigs (Matsui et al., 1996), chicks (Wedekind et al., 1992), sheep (Rojas et al., 1995), and dogs (Lowe et al., 1994a, b). However, researches (Hill et al., 1986; Pimentel et al., 1991) with pigs and chicks, respectively, indicated no differences in Zn bio-availability between organic and inorganic Zn sources. There was a big difference in the results of previous research. Currently, the mechanism

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of action of organic zinc (as well as organic trace element) is a "black box". The nutrient metabolism of organic zinc in digestive tract and intestinal wall has not been explained, which may be one of the reasons for inconsistent reports on the behavior of organic zinc.

Micro-X-ray fluorescence (μ-XRF) mapping is a non-destructive characterization technique that permits the two-dimensional imaging of the quantity and distribution of different elements in an inhomogeneous sample with ppm detectability. The use of capillary optics reduces the beam size and thus improves the spatial resolution (Pinakidou et al., 2007). On the other hand, μ-XRF directly gives elemental distributions without any special sample preparation.

The application of X-ray absorption fine structure (XAFS) technique in micron regions of samples-so called micro-XAFS (μ-XAFS) is getting attention in various fields (Heald et al., 1999). XAFS spectrometry is capable of non-destructive speciation of metals in solid samples, even if the element of interest is present in low concentrations (0.1%) (Osán et al., 2010). XAFS technology is not simply used to study the structure of crystalline matter or a material's electronic structure. It also is particularly sensitive to the chemical combination state and absorption geometry of atoms (Yoshimura et al., 2000). The X-ray absorption spectrum (XAFS) is typically divided into two regimes: X-ray absorption near-edge spectroscopy (XANES) and extended X-ray absorption fine-structure spectroscopy (EXAFS). Though the two have the same physical origin, this distinction is convenient for the interpretation. XANES is strongly sensitive to formal oxidation state and coordination chemistry (e.g., octahedral, tetrahedral coordination) of the absorbing atom, while the EXAFS is used to determine the distances, coordination number, and species of the neighbors of the absorbing atom (Matthew, 2004). This technology can not only determine trace element quality and quantity but can also detect combined elements and oxidation state (Ide-Ektessabi et al., 2002). Christensen et al. (2004) reported that using XANES spectroscopy can determine selenium oxidation states in animal mineral supplements. Therefore, the objective of the present experiment was to utilize μ-XRF and μ-XAFS technique for high-resolution, chemical-state-specific zinc assay in the duodenal wall to determine the change of zinc quantity, distribution and chemical state into intestinal wall using different zinc sources by technology of in situ ligated intestinal loops in broilers.

2. Materials and methods

2.1. Zn sources

In our experiment, reagent-grade Zn sulfate ($ZnSO_4 \cdot 7H_2O$; Tianjin Bodi Chemical Industry Co., Ltd., Tianjin, China) was used with Zn concentrations of more than 40.03%. Zn-methionine (ZnMet; Guangzhou Tanke technology Co., Ltd., Guangzhou, China) was also used with Zn concentration of more than 17.50%. Zn-Lysine (ZnLys; Guangzhou Tanke technology Co., Ltd., Guangzhou, China) also employed with Zn concentration of 10.50%. Zn content of Zn sources was measured by AAS.

2.2. Experimental design

The experimental design was approved by the Ethical Committee of the Veterinary Faculty of Northeast Agricultural University (China). Prior to the experiment, the broiler house was disinfected with white wash and fumigated with formalin gas, and drinkers were properly disinfected with 5% $KMnO_4$ solution, cleaned with water and dried under direct sunlight.

45 newly hatched healthy (1-d-old) commercial male broilers were used in this experiment and managed according to guidelines. All birds were randomly housed in stainless steel suspended cages with fiberglass feeders and plastic waterers. The birds were allowed ad libitum access to feed and tap water. The chicks were fed a corn–soybean meal basal diet (90.50 mg/kg, Table 1) from day 1 to 21 but were fed a semipurified diet (12.51 mg/kg, Table 1) after day 21 to deplete the body zinc stores. All other nutrients in these diets met or exceeded NRC (1994)

Table 1
Composition of diets for 1–21 and 22–28-d-old broilers

Items	Content	
	Basal diet (day 1 to 21)	Semipurified diet (day 22 to 28)
Ingredient (%)		
Corn	54.65	
Soybean meal	34.82	
Fish meal	3.5	
DL-Met	0.18	
Corn starch		66.00
Casein		23.00
Cellulose		5.01
Soybean oil	3.60	1.50
Calcium carbonate [a]	1.26	1.50
Calcium hydrogen phosphate [a]	1.30	1.12
Sodium chloride[a]	0.30	0.30
Micronutrients	0.39 [b]	1.57 [c]
Energy and nutrient composition [d]		
ME (MJ/kg)	12.58	13.22
CP (%)	21.66	19.33
Lys (%)	1.30	1.69
Met (%)	0.57	0.62
Met+Cys (%)	0.95	0.72
Calcium (%)	1.04	0.90
Nonphytate phosphorus (%)	0.45	0.40
Zinc (mg/kg)[e]	90.50	12.51

[a] Feed grade before day 21 (21-d-old) and reagent grade after day 21.

[b] Provided the following per kilogram of diet: vitamin A, 13,500 IU; vitamin D_3, 3600 IU; vitamin E, 33 IU; vitamin K, 6 mg; vitamin B_1, 4.5 mg; vitamin B_2, 10.5 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.03 mg; niacin, 60 mg; folic acid, 1.8 mg; biotin, 0.165 mg; choline, 700 mg; Cu, 8 mg; Zn, 60 mg; Mn, 100 mg; Fe, 80 mg; I, 0.35 mg; Se, 0.15 mg.

[c] Provided the following per kilogram of diet: vitamin A, 13,500 IU; vitamin D_3, 3600 IU; vitamin E, 33 IU; vitamin K, 6 mg; vitamin B_1, 4.5 mg; vitamin B_2, 10.5 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.03 mg; niacin, 60 mg; folic acid, 1.8 mg; biotin, 0.165 mg; choline, 700 mg; K, 3000 mg; Mg, 600 mg; Cu, 8 mg; Mn, 100 mg; Fe, 80 mg; I, 0.35 mg; Se, 0.15 mg.

[d] Nutrient composition is calculated value except zinc content.

[e] Determined values by atomic absorption spectrometry.