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Short communication

Effect of zinc source on the expression of ZIPII transporter genes in Guanzhong dairy goats

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ABSTRACT

The expression and function of the zinc transporter genes SLC39A1, SLC39A2, and SLC39A3 in the Zrt/Irt-like proteins (ZIP)II subfamily have been reported in monogastric but not ruminant animals. Thus, the response of these three ZIPII genes to different sources of zinc in ruminants was examined in goats in the present study. A total of 18 Guanzhong dairy goats were randomly divided into three groups of 6 goats each. The goats were fed one of three diets. The control group received a basic diet with no additional zinc. The second and third groups ate the same diet but with 60 mg/kg of zinc sulfate or zinc amino acid chelate (Zn-AA) complex added, respectively. After 7 days of treatment, the goats were sacrificed, 23 tissue samples were collected from each goat, and the expression and distribution of the ZIPII genes were determined with PCR analyses. In addition, seven tissues relevant to digestion or metabolism were examined using real-time PCR to identify the responses of the three genes to different zinc sources. The results showed that the expression of SLC39A1-3 mRNA could be detected in 21 of the 23 tissue types examined in the dairy goats, with no expression detected in heart or testis. The levels of expression for the three genes in the tissues relevant to digestion or metabolism were tissue specific and varied with the zinc source, indicating that the source of zinc affects the SLC39A1-3 mRNA expression.

1. Introduction

Zinc is a trace nutrient indispensable for life. More than 300 metalloenzymes of six major functional classes require zinc as a key structural component or as a cofactor (Vallee and Auld, 1990). As an important component of zinc finger domains, zinc regulates DNA replication, RNA transcription, and genes expression (Gaither and Eide, 2001). The absorption mechanism of zinc transport in cell and the transporter gene families has been described (Kambe et al., 2004; Hill and Link, 2009; Fukunaka and Kambe, 2010). In the animal feed industry, there are two main forms of zinc additives, organic and inorganic. Although

Abbreviations: CDF, cation diffusion facilitator; CP, crude protein; DM, dry matter; ME, metabolizable energy; NRC, national research council; PCR, polymerase chain reaction; SLC39A1, SLC39A2, and SLC39A3, gene codes for ZIP1, ZIP2, and ZIP3; TMR, total mixed ration; ZIP, Zrt/Irt-like proteins; Zn-AA, zinc amino acid chelate; $ZnSO_4$, zinc sulfate.

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Table 1
Nutrient composition and components in the basic diet (DM basis).

Ingredient	g/kg	Component	g/kg
Leymus chinensis hay	600	ME[b] (MJ/kg)	9.25
Alfalfa hay	100	DM	900.60
Corn	135	CP	114.60
Soybean meal	22	Zn[c] (mg/kg)	16.30
Cotton meal	48	Ca	3.80
Wheat middling powder	10	P	2.50
Corn DDGS	48	Ca/P	1.52
Palm meal	18	Na/K	1.60
Dibasic calcium phosphate	6		
Salt	3		
Premix[a]	5		
Limestone meal	0.30		
Sodium bicarbonate	5.40		

[a] The premix provided the following diet (per kg): VA 1, 620,000 IU; VD_3 324,000 IU; VE 540 IU; VK_3 150 mg; $FeSO_4 \cdot 7H_2O$ 170 mg; $CuSO_4 \cdot 5H_2O$ 70 g; $MnSO_4 \cdot 5H_2O$ 290 g; $CoCl_2 \cdot 6H_2O$ 510 mg; KI 220 mg; Na_2SeO_3 130 mg.
[b] ME, DM, CP were calculated according to the values provided in the Feed Database in China (2011).
[c] The Zn, Ca, P, Na, and K levels in the basic diet were measured values.

studies examining zinc digestive mechanisms vary widely (Evans et al., 1975; Tacnet et al., 1993; Vallee and Falchuk, 1993; Yu et al., 2007), a common understanding is that zinc can only be transformed into an organic form combined with protein for absorption into intestinal mucosal cells and eventual transport into body tissues (Lönnerdal, 2000). On the intestinal epithelial membrane, zinc transport proteins can be categorized into two families: the Zrt/Irt-like proteins (ZIP) protein family and the cation diffusion facilitator (CDF) protein family (Fukunaka and Kambe, 2010; Liuzzi and Cousins, 2004). Both families play important roles in zinc transport, with ZIP family proteins transporting zinc into the cytoplasm and CDF family proteins transporting zinc out of the cytoplasm. These two types of zinc transport are balanced under stable physiological conditions to maintain cellular zinc at specific concentrations but are varied in different tissues of the body and under diverse physiological conditions. Currently, there are at least 14 types of proteins known in the ZIP family in mammals, and these are cataloged into four subfamilies: I, II, LIV-1, and gufA. ZIPII protein family members are significant and include three members ZIP1, ZIP2, and ZIP3 with genes encoded by SLC39A1, SLC39A2, and SLC39A3 (SLC39A1-3), respectively (Kambe et al., 2008). These genes have been identified and shown to have a wide distribution in most tissues or organs (Andrews, 2008; Huang et al., 2006). Although the expression of these genes helped to illustrate the mechanisms of zinc absorption in rodents (Huang et al., 2006; Kambe et al., 2008) and poultry (Huang et al., 2008), the effect of different zinc sources on the expression of these genes in ruminants is scarce. Previously published papers have examined zinc function in ruminants involved in growth, nutrient utilization, the immune response, and carcass traits (Garg et al., 2008; Huerta et al., 2002; Mandal et al., 2007); however, the gene response was not examined, and thus, the characteristics of the response of ZIPII genes to various zinc sources remains unclear. Exploring the ZIPII gene responses to various zinc sources in ruminants will clarify the mechanisms for intracellular changes and will enrich the knowledge of the molecular mechanisms for zinc absorption and transportation. Therefore, the present study examined the expression and distribution of mRNA for SLC39A1-3 in dairy goats fed two sources of dietary zinc and identified the differences in the molecular mechanisms for these genes.

2. Materials and methods

2.1. Animals, experimental design, and treatments

A total of 18 adult Guanzhong dairy goats (40–45 kg of body weight; 2.5–3.0 years of age) were randomly divided into three groups (equal numbers of males and females in each group). Goats 1–6 were in the control group; goats 7–12 were in the group administered zinc sulfate ($ZnSO_4$ group); goats 13–18 were in the group administered zinc amino acid chelate complex (Zn-AA group). All goats were individually housed in dedicated iron cages with enough space for the animals to move around inside. All of the animals received care according to the *Guide for the Care and Use of Laboratory Animals* by the Chinese Academy of Sciences. Basal rations were formulated to meet the nutrient requirements for goats according to NRC guidelines (2007), except for the zinc additive. The chemical and ingredient composition in the basic diet is listed in Table 1. Analyses of the Zn, Ca, Na, and K in the diet were conducted using atomic absorption spectrophotometry (Z-2000, Hitachi, Japan), and the analysis of P was conducted with colorimetry (T6, Pgeneral, Beijing). ME, DM and CP in the diets were determined according to AOAC (1990) procedures.

The control group was fed the basal diet, and the $ZnSO_4$ and Zn-AA groups were assigned $ZnSO_4$-supplemented and Zn-AA-supplemented diets, respectively. Zinc-supplemented diets were prepared by adding calculated amounts of $ZnSO_4$ (produced in the Tianjin chemical plant, China; ≥98% purity, ≥22.5% zinc) or Zn-AA (donated by Chengdu Aohe Biotechnology Co., LTD. China; complex amino acids, ≥92% chelating rate, ≥25% AA, ≥10% zinc) to the basic diet (the original zinc already in the basal feedstuff was ignored), modifying the zinc level to 60 mg/kg (DM). All the diets were supplied as total mixed ration (TMR). Feed and demineralized water were provided *ad libitum* throughout the experiment. The duration of the feeding

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