The influence of administration of different doses of arginine and lysine coupled with zinc in the lactating ewes on the zinc concentration in milk

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Summary

Zinc (Zn) plays an essential role in the human and animal body system. Zinc deficiency might cause many disorders, so it is important to provide a sufficient amount of this microelement in both animals and humans. One of the most important sources of Zn is milk. The purpose of this study was to determine whether Zn can pass through the mammary epithelial cell into milk by a co-transport system with amino acid in the lactating ewes. This experimental research included 54 lactating ewes collected from the Agriculture-Jahad Organization of Tehran Province, that were allocated into 9 groups as follows: groups which received inorganic form as Zn chloride (1, 2 and 4 mg/kg), and groups which received organic Zn with different doses of arginine or lysine (1, 2 and 4 mg/kg) in chelate form. Milk samples were taken 30 min before and 6 h after intravenous injection of Zn preparations. Zinc concentration in milk was measured using the flame atomic absorption spectrometric method. There were no significant differences ($P>0.05$) between groups in Zn concentration of the milk before administration of inorganic or organic Zn. Data analysis showed that administration of inorganic Zn caused a significant increase of Zn concentration in the milk in a dose dependent manner. While administration of arginine or lysine with Zn in chelate form caused a significant decline in Zn concentration of milk compared to inorganic Zn in a dose dependent manner. According to our results, administration of different forms of Zn influenced the Zn concentration in milk.

Key words: Arginine, Ewe milk, Lactation stage, Lysine, Zinc

Introduction

Zinc (Zn) is one of the essential trace elements which binds to different proteins and enzymes in animals and human that are involved in a broad range of physiological processes. The decrement or increment in concentration of Zn might cause many disorders, so preventing trace mineral deficiencies has long been recognized as important in the maintenance of production, reproduction, and health (Nemec *et al*., 2012). One of the most important sources for Zn is the dietary supplementation with milk. In recent years, many attempts have been made in order to provide sufficient status of this microelement in both animals and humans. Traditionally, the range of supplements that are used for elevating Zn concentration, contain mostly inorganic Zn as sulfate salts (Pechova *et al*., 2009). Organic forms of Zn including metal amino acid chelates, metal complexes, metal methionine hydroxy analog chelates, metal proteinates, and metal propionates have been developed to increase intestinal absorption and mineral bioavailability (Nemec *et al*., 2012). Hatfield *et al*. (2001) demonstrate that supplementing mature ewes with complexed organic minerals resulted in higher concentrations of Zn than inorganic source in the liver. Another study suggested that ewes supplemented with Zn in the diet from Zn-methionine had a higher Zn concentration in different tissues than ewes that received Zn-sulfate (Pal *et al*., 2010).

Zinc cell uptake might be under different mechanism such as through its specific channel or co-transporting system. Previous study showed that in humans, 0.5-1 mg of Zn per day is transported through the mammary gland into milk (King, 2002) and Zn transportation into milk is assumed to be an active process (Kelleher and Lonnerdal, 2003). In cow milk, Zn primarily binds to casein and, to a small extent, to citrate. Almost 90% of Zn binds to casein in mature milk in contrast to just 60% in the colostrum (Kincaid and Cronrath, 1992). In casein, Zn mainly binds to colloid calcium phosphate in casein micelles (Silva *et al*., 2001). De la Fuente *et al*. (1997) reported that 87.5% of Zn is contained in the micellar fraction of goat's milk.

In the mammary gland, the mechanism of Zn uptake is not quite clear and there is little information about factors that influenced the concentration of Zn in ewe's milk. Therefore, the goal of this study was to determine whether Zn can pass through the mammary epithelial cell into milk by a co-transport system with amino acid in the lactating ewes.

Materials and Methods

Animal and experimental design

In this experimental study, 54 lactating ewes which were in the third-fourth month of the first lactation were collected from the Agriculture-Jahad Organization of Tehran. This study was approved and conducted in accordance with the regulations of the Animal Ethics Committee of Animal Science and Veterinary Faculty of Tehran University, Iran. Ewes were assigned into 9 groups and each group included 6 ewes. Groups which received Zn in inorganic form as Zn chloride ($ZnCl_2$ 1; Merck, Germany) in different doses (1, 2 and 4 mg/kg), and groups which received organic Zn (4 mg) with different doses of arginine (1, 2 and 4 mg; Merck, Germany) or lysine (1, 2 and 4 mg/kg; Merck, Germany) in chelate form. Zinc preparations were administered individually by intravenous (*i.v*) injection in all treatment groups.

Sample collection and analysis

Milk samples were taken 30 min before and 6 h after administration. Milk Zn concentrations were measured using the flame atomic absorption spectrometric (F-AAS) method and the Analytical CTA-2000 (Chemtech, UK) device. The milk was ashed by the wet process with hydrogen dioxide and nitric acid addition (2 ml milk + 1 ml H_2O_2 + 2 ml HNO_3) using the microwave digestion technique in the MLS-1200 (Milestone, Italy) microwave oven. At the end Zn concentration in milk was expressed as ppm using calibration curve.

Statistical analysis

Statistical analysis was performed using SPSS software (version 22; SPSS Inc., Chicago, IL., USA). The results were statistically assessed using one-way ANOVA analysis of variance. Following a significant p-value, *post hoc* analysis (Tukey's test) was performed for multiple comparisons. The significance between before and after of each corresponding group was assessed with the help of the paired sample t-test. The results were displayed as a mean value with standard error of mean (mean±SEM). P<0.05 was considered to be significant. Graphs' drawing was done using Microsoft Excel software.

Results

Influence of administration of inorganic Zn on milk Zn concentration

The concentrations of inorganic Zn in milk are shown in Fig. 1. As shown at the start of the experiment, there were no differences between groups in the concentration of Zn in milk before intervention [0.308 ± 0.027 ppm for Zn (1 mg/kg), 0.343 ± 0.049 ppm for Zn (2 mg/kg), and 0.318 ± 0.04 ppm for Zn (4 mg/kg)], however, we found significant rise in the average concentrations of Zn in milk at doses 1, 2 and 4 mg/kg (0.739 ± 0.031 ppm, P<0.05, 0.986 ± 0.005 ppm, P<0.01, and 1.332 ± 0.043 ppm, P<0.001, respectively) 6 h after Zn administration.

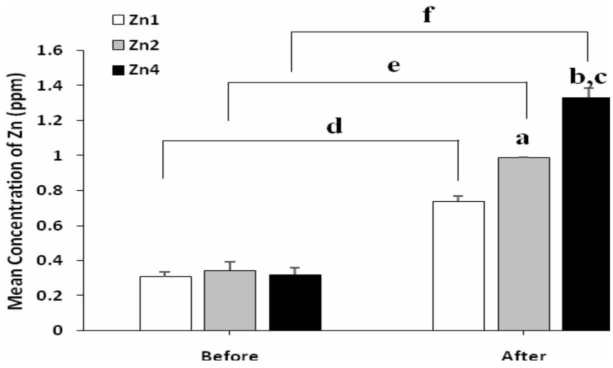

Fig. 1: Mean zinc (ppm) concentration in milk before and after administration of Zn (1, 2 and 4 mg/kg) in treatment groups. Data expressed as mean ± SEM (n=6). a: P<0.01 and b: P<0.001 vs. Zn (1 mg/kg), c: P<0.001 vs. Zn (2 mg/kg), d: P<0.05, e: P<0.01, and f: P<0.001 as compared with corresponding before and after groups

Influence of administration of arginine and Zn on milk Zn concentration

Figure 2 shows the concentrations of Zn in milk before and after administration of arginine and Zn in chelate form. No significant differences were seen in Zn concentration of milk in 4 mg/kg Zn + 1 mg/kg arginine (0.266 ± 0.02 ppm), Zn + 2 mg/kg arginine (0.24 ± 0.031) and 4 mg/kg Zn + 4 mg/kg arginine (0.256 ± 0.021 ppm) groups at the start of the experiment. Administration of 4 mg/kg Zn with 1 and 2 mg/kg arginine significantly increased the Zn concentration of milk to 0.795 ± 0.044 ppm, and 0.629 ± 0.035 ppm, respectively (P<0.01), while 4 mg/kg Zn + 4 mg/kg arginine had no effect on Zn concentration of milk. However, significant decline in milk Zn concentration was observed in groups which received arginine at different doses and Zn (4 mg/kg) in comparison with inorganic Zn (4 mg/kg) (the differences are shown in Fig. 2).

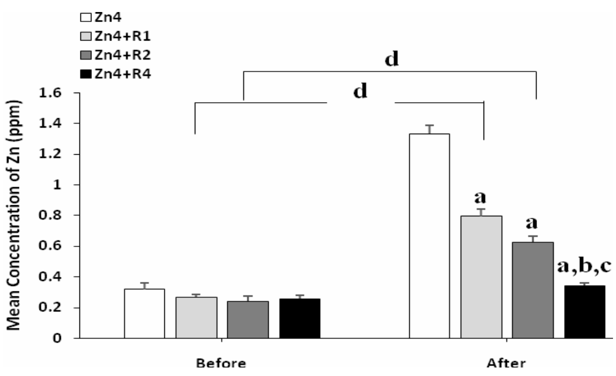

Fig. 2: Mean zinc (ppm) concentration in milk before and after administration of arginine (R) (1, 2 and 4 mg/kg) coupled with Zn (4 mg/kg) in treatment groups. Data was expressed as mean ± SEM (n=6). a: P<0.001 vs. Zn4, b: P<0.001 vs. Zn4 + R1, c: P<0.001 vs. Zn4 + R2, and d: P<0.01 as compared with corresponding before and after groups

Influence of administration of lysine and Zn on milk Zn concentration

Figure 3 shows the concentrations of Zn in milk before and after administration of lysine and Zn in chelate form. As shown there were no differences between groups in the concentration of Zn in milk for 4 mg/kg Zn plus 1 mg/kg lysine (0.248 ± 0.034 ppm) for 4 mg/kg Zn plus 2 mg/kg lysine (0.322 ± 0.029 ppm) and for 4 mg/kg Zn plus 4 mg/kg lysine (0.238 ± 0.024 ppm) groups, at the start of the experiment. Statistical analysis shows that average concentrations of Zn in milk of different groups were affected after administration. Significant rise has been found between groups in average concentrations of Zn in milk at doses 1 and 2 mg/kg lysine plus Zn (4 mg/kg) (0.628 ± 0.027 ppm, $P<0.05$; 0.539 ± 0.064 ppm, $P<0.01$, respectively) in comparison with before administration, but no significant rise was observed in group which received lysine (4 mg/kg) plus Zn (4 mg/kg) before and after administration. However, significant decline in milk Zn concentration in groups which received lysine at different doses and Zn (4 mg/kg) in comparison with inorganic Zn (4 mg/kg) was observed (the differences are shown in Fig. 3).

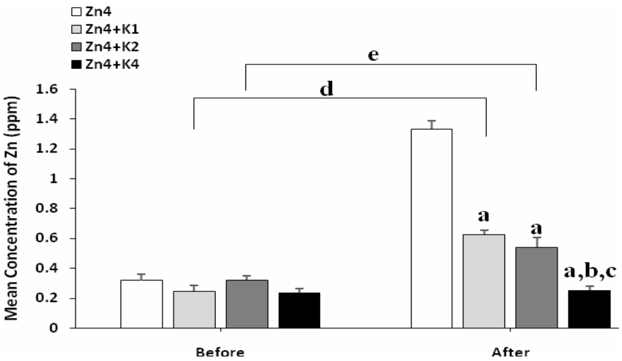

Fig. 3: Mean zinc (ppm) concentration in milk before and after administration of lysine (K) (1, 2 and 4 mg/kg) coupled with Zn (4 mg/kg) in treatment groups. Data are expressed as mean ± SEM (n=6). a: $P<0.001$ vs. Zn4, b: $P<0.001$ vs. Zn4 + K1, c: $P<0.05$ vs. Zn4 + K2, d: $P<0.05$, and e: $P<0.01$ as compared with corresponding before and after groups

Discussion

Ewes supplemented with arginine- and lysine-Zn both had higher milk Zn concentrations compared with corresponding before treatment groups. Interestingly, supplementation with an inorganic form of Zn showed a significant increase when compared with organic Zn. In other words, supplementation of Zn with organic complex did not significantly increase the excretion of Zn into milk (6 h after administration) as compared with inorganic Zn. Studies focusing on the relation between milk Zn concentration and the level of supplementation are scarce and were mostly realized in the dairy cows and the results show a very different conclusion. Pechova *et al.* (2009) did not detect an increase of Zn concentration in milk of goats with 500 mg organic Zn for a period of 28 days or in milk of dairy cows with supplementation of 440 mg Zn-chelate (Pechova *et al.*, 2006). Also, Kirhgessner *et al.* (1994) did not bring any conclusive proof of an increase in milk Zn concentration either, after supplementation of 2.2 g Zn-methionine. On the other hand, Strusinska *et al.* (2004) revealed an increase in colostrum Zn concentration by 3-10% when supplementing organic microelements, and Benuska *et al.* (1991) observed an increase in cow milk Zn concentration after intramuscular administration of Zn. Hermansen *et al.* (1995) found that feeding a high concentration of Zn only tended to increase the concentration of Zn in whole milk but significantly increased the concentration in the cream fraction. The study of Wiking *et al.* (2008) indicate that the plasma and milk Zn levels are increased by saturating lipid in the diet and thereby, dietary fat might facilitate the transfer of dietary Zn into plasma and milk.

In our study, supplementation with organic Zn did not significantly increase the level of milk Zn concentration when compared with inorganic Zn. For interpretation of this result, studies show that, when an amino acid chelate or chelate/complex is delivered to the tissue or organ as an intact molecule containing both the essential metal and the essential amino acid ligand, the complete amino acid chelate or chelate/complex molecule may be able to participate in certain metabolic activities in a balanced and somewhat synergistic relationship, which would then cause increased retention of both components. If a high amount of a certain mineral from an ionized source is delivered to this same "metabolic center" without a corresponding amount of an essential (for the metabolic process) amino acid, the excess metal will be endogenously excreted until a balance (for metabolic activity) is achieved between the metal and whatever amount of amino acid was available (Ashmead, 2012).

Zinc is essential for milk production (Kirchgessner and Weigand, 1982). Yet, our study demonstrated that ewes receiving these minerals from inorganic metal salt sources did not retain as much of these required minerals in their tissues as did other groups provided the exact same minerals in the amino acid-chelated form. One of the mediums for endogenous excretion of excess minerals is milk (Miller, 1975). When the dairy animals fed the inorganic salts of manganese, zinc, and copper, they endogenously excrete significantly more of these minerals into their milk, resulting in less milk and milk component production because the minerals are not retained and used in the tissues for milk production. The metal amino acid chelates are held in the tissues in greater quantities (less excretion into the milk) and influence increased metabolism in the body (greater milk and milk component production) (Ashmead, 2012).

Summing up, the administration of different forms of Zn influenced the concentration of Zn in milk. Inorganic forms of Zn caused a significant increase of Zn concentration in the milk in the dose dependent manner. In contrast, organic form was not as effective as inorganic form in increment of milk Zn concentration compared to inorganic Zn in the dose dependent manner.

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Conflict of interest

The authors declare that they have no competing interests in this work.

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