Original Research

Evaluation of Zn Bioavailability and Metallothionein Expression by RT-PCR in Zinc Sulphate and Zinc Proteinate Fed Chickens

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Abstract

The present study was conducted to evaluate the bioavailability of different forms and concentration of Zinc (Zn) incorporated into diets of chicken and to find out Metallotheionein (MT) expression by Real time Polymerase Chain Reaction (RT-PCR). In this study broiler birds were fed with corn-soya based basal diet (BD) as control (T_1), BD with $ZnSO_4$@ 80 ppm (T_2) and BD with 40 and 80 ppm of Zn-proteinate (T_3 and T_4) respectively, for a period of 42 days. At day 42, six birds from each treatment were slaughtered, liver and duodenal scrapings were collected for MT expression, whereas tibia and serum were collected for Zn estimation. On comparison of treatments and control group for Zn bioavailability and MT mRNA expression, treatment groups showed significantly higher levels of Zn in tibia and serum and also up regulation of MT mRNA. But, birds fed with Zn-proteinate exhibited MT mRNA expression significantly greater than all other treatments, suggesting that Zn-proteinate form is more bioavailable than $ZnSO_4$. Subsequent commercial trails with reduced (50 %) mineral inputs via Zn-proteinate revealed equal performance characteristics as compared to inorganic Zn feeding at 100 % level.

Key words: Bioavailability, Metallotheionein, RT-PCR, Zn-proteinate, Zinc

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Introduction

In the past ten years feed industry has focused on supplementation of organic trace minerals in livestock and poultry diets, owing to the lack of trace minerals in the naturally available feed ingredients. One such mineral is Zinc (Zn) which is essential for growth, skeletal development and immune competence in

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chickens (Hudson *et al.*, 2004). However, there are varying results reported regarding bioavailability of organic products relative to traditional inorganic forms of the elements (Haung *et al.*, 2013). It has been documented that organic Zn supplementation in broiler chicken was more effective in promoting growth with less interaction with other minerals than Zn sulphate (Ao *et al.*, 2011). However, the relative bioavailability of this Zn-proteinate has not been experimentally verified by using gene expression studies. Serum and bone Zn accumulation is generally used to measure the bioavailability of different Zn sources. Metallothionein (MT), a Zn-binding protein, plays a critical role in Zn transport and storage and hence MT expression is widely used as an indicator of the Zn status and also to evaluate the bioavailability. Liver and intestine MT mRNA level has been more sensitive than tibia Zn concentration in differentiating the differences in the bioavailability of various Zn sources for chickens. Therefore, the objective of this study was to investigate the bioavailability of Zn sources fed to chickens and metallotheionein (MT) expression by Real Time Polymerase Chain Reaction (RT-PCR).

Materials and Methods

The present study was performed in the Department of Poultry Science, Madras Veterinary College, TANUVAS (Chennai, India). Day old Cobb chicks (120) were randomly allotted to 3 replicate with 10 chicks each and these replicate were distributed randomly to 4 dietary treatments. A corn-soybean meal diet without Zn supplementation was used as a basal diet (T_1- control). Basal diet with inorganic Zn ($ZnSO_4$) at 80 ppm (T_2) and organic Zn (Zn-proteinate) at 40 and 80 ppm (T_3 and T_4), respectively forms the treatment groups. The birds were raised in brooder cum grower cages under uniform management for a period of 42 days. At the end of 42thday, two broiler chickens from each replicate will be slaughtered, liver and duodenal scrapings were collected to measure the MT mRNA expression by RT-PCR whereas, tibia and serum for estimation of Zn concentration.

Blood samples were collected from the brachial vein and processed for serum separation. Serum samples were stored at -20°C until further analysis. After slaughter the bone (left tibia) were collected and adhering muscles were removed manually. Then these bones were dipped in 10% sodium hydroxide (NaOH) solution for 5 min to remove the adhering fine and soft tissue. These bones were dried in hot air oven overnight. De-fattening of dried bones was done with diethyl ether and petroleum sprit. Dried bones were ashed at 650˚C for 4 h in muffle furnace as per the method of AOAC (2000). The Zn content in the tibia and serum was estimated by using Atomic absorption spectrophotometer (Perkin Elmer Analyst 400).The primers used for RT-PCR to amplify MT gene along with endogenous control β-actin gene of *Gallus gallus domesticus* and the cyclic conditions are listed in Table 1 and 2. The RT- PCR was carried out using SYBR green based method for the zinc specific gene in eppendroff qPCR master cycler using SYBR Premix Ex Taq (Sigma, Invitrogen, USA). The RT-PCR data were analysed using the $2^{-\Delta\Delta ct}$

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method reported by Livak and Schmittgen (2001) and β-actin was chosen as a reference to normalise the expression level of MT mRNA.

Table 1: List of oligonucleotide primers for Real time PCR

Primers	Sequence (5' to 3')	Product Size
MT-FP	AAG GGC TGT GTC TGC AAG GA	163 bp
MT-RP	CTT CAT CGG TAT GGA AGG TAC AAA	
β-actin-FP	GAG AAA TTG TGC GTG ACA TCA	152 bp
β-actin -RP	CCT GAA CCT CTC ATT GCC A	

Table 2: The cyclic conditions used for RT-PCR

Step	Temperature	Time	
Initial denaturation	95°C	2min.	
Denaturation	95°C	10 sec	Cycling stage
Annealing	62°C	20 sec	40 cycles
Extension	72°C	45 sec	
Melt curve	Default settings		

Results and Discussion

Tibia and Serum Zn

The supplementation of inorganic and organic forms of Zn in both control and treatment birds and their concentration of Zn in tibia and serum are presented in Table 3.

Table 3: Tibia and serum Zn concentration (ppm) Mean (±SE) in broiler chickens fed with inorganic and organic zinc

Treatments(in ppm)	Tibia Zn (ppm)	Serum Zn (ppm)
T1Control	$59.45^{a} \pm 1.82$	$0.96^{a} \pm 0.08$
T2$ZnSO_4$ - 80	$112.48^{b} \pm 1.38$	$1.96^{b} \pm 0.08$
T3Zn-Pro - 40	$113.01^{b} \pm 2.31$	$2.23^{b} \pm 0.11$
T4Zn-Pro - 80	$111.25^{b} \pm 2.52$	$2.03^{b} \pm 0.08$
F value	162.35	39.38
Significance	** Highly significant (P<0.01)	

Means bearing different superscripts within the same column differ significantly (P<0.01)

The mean tibia and serum Zn concentration was highly significant (P<0.01) in the treatment groups when compared to control groups. The mean tibia Zn concentration (ppm) was 112.48, 113.01 and 111.25 in the treatment groups (T_2, T_3 and T_4) as compared to 59.45 in the control. Whereas, serum Zn concentration (ppm) was 1.96, 2.23 and 2.03 in the treatment groups (T_2, T_3 and T_4) as compared to 0.96 in the control. Bones have been established as functional reserve of Zn in broiler chickens, which can be used during Zn deficiency (Suttle, 2010).The findings of the present study are in agreement with Sunder *et al.* (2008) who have noticed a linear increase in Zn deposition in bone with respect to Zn supplementation in the diets of

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broilers. Similarly, Mandal *et al.* (2012) reported that tibial Zn concentration was increased (P<0.01) in birds supplemented with Zinc proteinate.

The findings of the present study are in agreement with Liu *et al.* (2013) who observed that birds fed with diets supplemented with Zn proteinate had higher (P<0.05) Zn concentrations in plasma and tibia ash than those fed diets supplemented with Zn sulphate. Tronina *et al.* (2007) who evaluated the Zn content in tibia bone for broiler chickens supplemented with inorganic (ZnO) and organic (Zn-glycine) zinc observed increased concentration of zinc in the Zn-glycine supplemented groups. Thus, it could be inferred from the results that birds supplemented with the organic Zn showed higher tibia and serum Zn concentration when compared to the inorganic Zn supplemented group and control. The mechanism for this might be explained by the antagonism occurring between Zn and other minerals (such as Cu) when included in inorganic forms as compared to inclusion of zinc in organic forms in diet.

MT mRNA Expression

The results of the present study showed a highly significant (P<0.01) up regulated expression of MT gene in organic and inorganic Zn treatments when compared with control. But, the expression in inorganic Zn was low when compared with organic source of Zn. Liver and intestinal MT mRNA expression for different treatment groups are shown in Fig. 1. The mean relative expression of MT mRNA in liver was 6.03, 6.15 and 6.03 in the treatment groups (T_2, T_3 and T_4) as compared to 1.0 in the control. Whereas, the mean relative expression of MT mRNA in intestine was 22.17, 38.74 and 46.47 in the treatment groups (T_2, T_3 and T_4) as compared to 1.0 in the control.

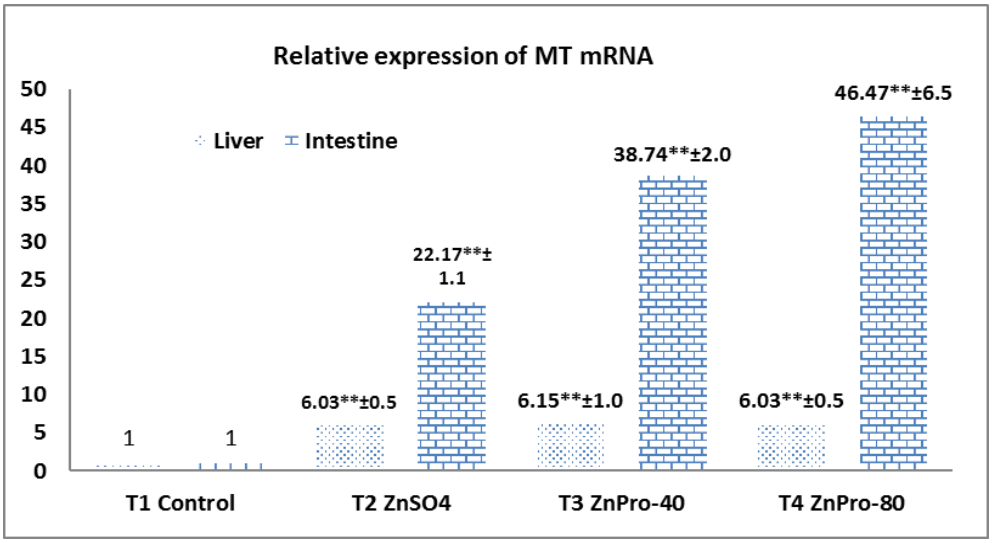

Fig.1: Mean (±SE) relative expression of MT mRNA in broiler chickens fed with inorganic and organic zinc

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MT is an important maintainer of the Zn pool of the organisms, and plays a protective role in antioxidant responses by scavenging free radicals, particularly the hydroxyl radical (Ruttkay-Nedecky *et al.,* 2013). Dietary Zn was reported to increase liver Zn and MT content (Wang *et al.,* 2012). In our study, the linear relationship between dietary Zn level and tissue Zn contents has been observed, which might be attributed to higher levels of dietary Zn supplementation. The findings of the present study are in agreement with Huang *et al*. (2009) who reported that Zn supplementation linearly increased MT expression in pancreas. Similarly, Cheng and Guo (2004) compared the inorganic Zn and Zn amino acid (ZnAA) chelates with MT expression and suggested that ZnAA complex enhanced MT synthesis in liver. Brooks *et al.* (2013) and Liu *et al.* (2013) reported that supplementation of organic Zn showed an increased MT mRNA expression when compared with $ZnSO_4$ and control.

Based on the results of the present study it was evident that when birds are fed with corn soya based diet, containing the dietary antagonist phytic acid and inorganic Zn, the Zn usually reacts with phytic acid and goes unutilized as Zn-phytate complex. Whereas, the increased bioavailability of chelated trace minerals is likely due to its reduced antagonistic reactions with other dietary constituents in the GI tract of the bird. Another possible reason is its chelation strength. The organic trace elements with the moderate chelation strength displayed the highest relative bioavailability, followed by elements with the strong chelation strength, and those with the weak chelation strengths were as available as their inorganic forms. Hence, Zn-proteinate with moderate chelation strength has higher bioavailability when compared with inorganic Zn.

Conclusion

Our study showed that organic source of Zn (Zn-proteinate) have relatively higher bioavailability which facilitates growth in the broilers. Attempts to utilize Zn protienate as feed supplement can be tried at large scale levels for efficient weight gain in the poultry birds.

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