

Original Research Article

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## Real Time PCR Based Expression of Metallothionein and Evaluation of Zn Bioavailability in Chickens Fed Zinc Oxide and Zinc Methionine

A. Varun<sup>1\*</sup>, N. Karthikeyan<sup>1</sup>, P. Muthusamy<sup>2</sup>, A. Raja<sup>3</sup> and S. Wilfred Ruban<sup>4</sup>

<sup>1</sup>Department of Poultry Science, Madras Veterinary College, Chennai, Tamil Nadu, India

<sup>2</sup>Post Graduate Research Institute in Animal Sciences, TANUVAS, Kattupakkam, Tamil Nadu, India

<sup>3</sup>Department of Microbiology, VCRI, Namakkal, Tamil Nadu, India

<sup>4</sup>Department of Livestock Product Technology, Veterinary College, Bengaluru, Karnataka, India

\*Corresponding author

### ABSTRACT

An experiment was conducted to investigate the bioavailability of different forms and concentration of Zinc (Zn) fed to chickens and Metallothionein (MT) expression by Real time Polymerase Chain Reaction (RT-PCR). In the present study broiler birds were fed with corn-soya based basal diet (BD) as control (T<sub>1</sub>), BD with ZnO 80 ppm (T<sub>2</sub>) and BD with 40 and 80 ppm of Zn-methionine (T<sub>3</sub> and T<sub>4</sub>) 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. When compared with control, all the treatment groups had higher levels of Zn in tibia and serum and also up regulation of MT mRNA significantly. But, birds fed with Zn- methionine exhibited MT expression significantly greater than all other treatments and suggesting that Zn-met form is more bioavailable than ZnO. Subsequent commercial trails with reduced (50 %) mineral inputs via Zn-Methionine revealed equal performance characteristics as compared to inorganic Zn feeding at 100 per cent level.

#### Keywords

Zinc,  
Bioavailability,  
Metallothionein,  
RT-PCR, Zn-  
Methionine.

#### Article Info

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### Introduction

Zinc (Zn) as a micro mineral is essential for growth, skeletal development, and immune competence in chickens (Hudson *et al.*, 2004). Inorganic Zn salts, such as sulphate and oxide, are generally supplemented in diets to meet the Zn requirement of chickens (Cao *et al.*, 2002). However, in the recent past Inorganic trace minerals (ITMs) have been replaced by organic trace minerals (OTMs) because of higher bioavailability and

considerable reduction in cost of mineral supplementation. Several researchers have conducted different trails with both organic and inorganic Zn supplementation and they have observed increased availability in organic forms of minerals (Yan Waldroup, 2006 and Ao *et al.*, 2011).

However, in some studies, no differences in bioavailability were observed between

organic and inorganic forms of Zn products (Pimentel *et al.*, 1991 and Haung *et al.*, 2009). These discrepancies might be related to the degree of chelation or complexation of the Zn ion to organic ligands.

It has been documented that Zn-methionine 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-methionine 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 for chicks. 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). Vencobb 400 chicks (n=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<sub>1</sub>-control). Basal diet with inorganic Zn (ZnO) at 80 ppm (T<sub>2</sub>) and organic Zn (Zn methionine) at 40 and 80 ppm (T<sub>3</sub> and T<sub>4</sub>), 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 42<sup>th</sup> day, 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. Defatting of dried bones was done with diethyl ether and petroleum spirit. 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  $\beta$ -actin gene of *Gallus gallus domesticus* and the cyclic conditions are listed in tables 1 and 2.

The RT-PCR was carried out using SYBR green based method for the zinc specific gene in eppendr off qPCR master cycler using SYBR Premix Ex Taq (Sigma, Invitrogen, USA). The real-time quantitative PCR data were analysed using the  $2^{-\Delta\Delta^{ct}}$  method reported by Livak and Schmittgen (2001), and  $\beta$ -actin was chosen as a reference to normalise the expression level of MT mRNA.

## **Results and Discussion**

### **Tibia and serum Zn**

The effect of inorganic and organic Zn supplementation along with control on the

tibia and serum Zn concentration of broiler chickens are presented in table 3. 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 106.94, 116.44 and 112.48 in the treatment groups (T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>) as compared to 59.45 in the control. Whereas, serum Zn concentration (ppm) was 2.07, 2.29 and 2.25 in the treatment groups (T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>) as compared to 0.96 in the control. Similar results have been reported by Sunder *et al.*, (2008) who have noticed a linear increase in Zn deposition in bone with respect to Zn supplementation in the diets of broilers.

The findings of the present study are in agreement with Tronina *et al.*, (2007) who evaluated the Zn content in tibia bone for broiler chickens supplemented with inorganic (ZnO) and organic (Zn-glycine) zinc and observed increased concentration of zinc in the Zn-glycine supplemented groups. Similarly, Bartlett and Smith (2003) observed that broilers supplemented with Zn showed a positive correlation of tibial Zn concentration with increasing concentration of Zn in the feed. 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 revealed 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 figure 1. The mean relative expression of MT mRNA in liver was 3.4, 6.1 and 3.3 in the treatment groups (T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>) as compared to 1.0 in the control. Whereas, the mean relative expression of MT mRNA in intestine was 15.4, 49.3 and 44.4 in the treatment groups (T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>) as compared to 1.0 in the control.

**Table.1** List of oligonucleotide primers for Real time PCR

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

**Table.2** The cyclic conditions used for RT-PCR

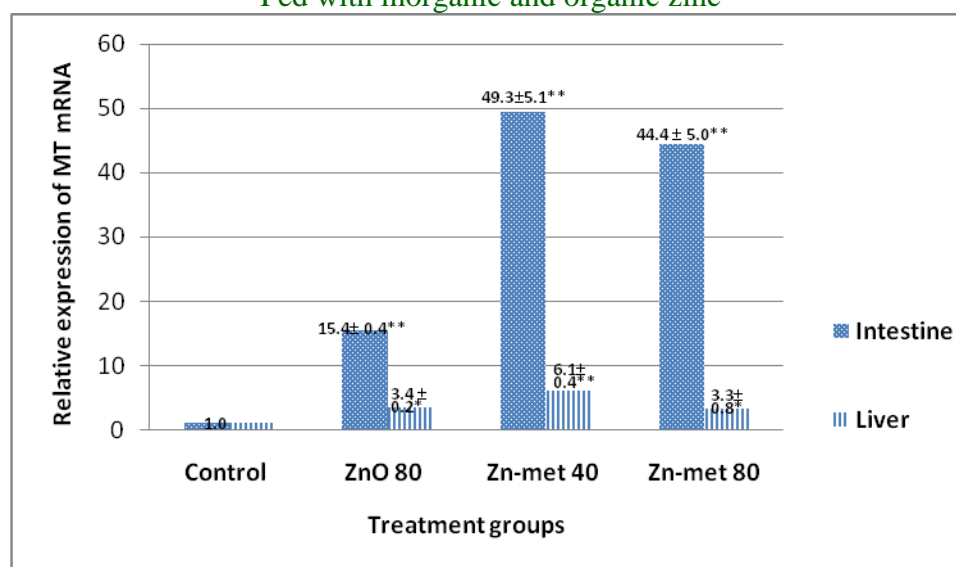
<b>Step</b>	<b>Temperature</b>	<b>Time</b>	
Initial denaturation	95°C	2min.	
Denaturation	95°C	10 sec	<b>Cycling stage 40 cycles</b>
Annealing	62°C	20 sec	
Extension	72°C	45 sec	
Melt curve	Default settings		

**Table.3** Mean ( $\pm$ SE) tibia and serum Zn concentration (ppm) in broiler chickens Fed with inorganic and organic zinc

Treatments(in ppm)	Tibia Zn (ppm)	Serum Zn (ppm)
<b>T1Control</b>	59.45 <sup>c</sup> $\pm$ 1.82	0.96 <sup>b</sup> $\pm$ 0.08
<b>T2ZnO - 80</b>	106.94 <sup>ab</sup> $\pm$ 0.47	2.07 <sup>a</sup> $\pm$ 0.02
<b>T3Zn-met - 40</b>	116.44 <sup>a</sup> $\pm$ 2.10	2.29 <sup>a</sup> $\pm$ 0.05
<b>T4Zn-met - 80</b>	112.48 <sup>ab</sup> $\pm$ 1.38	2.25 <sup>a</sup> $\pm$ 0.04
<b>F value</b>	<b>55.007</b>	<b>33.046</b>
<b>Significance</b>	** Highly significant (P<0.01)	

Means bearing different superscripts within the same column differ significantly (P<0.05)

**Fig.1** Mean ( $\pm$ SE) relative expression of MT mRNA in broiler chickens Fed with inorganic and organic zinc



\*\* - Highly significant (P<0.01) \* - Significant (p< 0.05)

The findings of the present study are in agreement with Huang *et al.*, (2007) 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. Cao *et al.*, (2002) and Liu *et al.*, (2013) reported that supplementation of organic Zn showed an increased MT mRNA expression when compared with ZnSO<sub>4</sub> and control. Brooks *et al.*, (2013) reported that relative bioavailability of Zn from organic Zn was much higher than Zn sulphate.

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, the Zn usually reacts with phytic acid and 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-met with moderate chelation strength have higher bioavailability when compared with inorganic Zn.

From our study it was concluded that organic source of Zn (Zn-met) have relatively higher bioavailability and supports the performance in the broilers. Furthermore, Zinc methionine was more effective than inorganic Zn in enhancing the MT mRNA expression in broilers.

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