Bioavailability of zinc sources in chickens determined via real time polymerase chain reaction (RT-PCR) assay for metallothionein

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Tissue zinc is used to define zinc availability, but deposition is far from the absorption site and only a fraction of absorbed zinc is deposited. Alternatively, zinc availability may be defined by quantifying expression of specific zinc-responsive genes in the small intestine. Metallothionein (MT) protein binds zinc and its mRNA expression is reported to reflect zinc status. A real time polymerase chain reaction (RT-PCR) assay was developed to measure MT mRNA expression in chickens fed titrated levels or sources of zinc. MT was inducible by zinc in small intestine, liver and kidney. Broilers were fed a milo-soy diet for 20 days, then were provided an unsupplemented corn-soy basal diet or the basal with 70ppm zinc from zinc oxide (ZO), a zinc amino acid complex (ZAAC) or MINTREX® Zn. Jejunum scrapings were collected after two days and assayed for MT expression. ZO and ZAAC failed to induce MT expression significantly above the basal. Birds fed MINTREX Zn exhibited MT expression significantly greater than all other treatments, suggesting that MINTREX Zn is more bioavailable than ZO or ZAAC. Subsequent commercial trials with reduced (50%) mineral inputs via MINTREX support equal live performance.

Keywords: zinc; bioavailability; metallothionein; RT-PCR; MINTREX

Introduction

Organic trace minerals (OTMs) are fed to provide a more bioavailable form of trace minerals than ITMs. Indeed, tissue mineral and slope-ratio type experiments have demonstrated the increased availability of organic forms compared to inorganic forms (Fly, et al., 1989; Paik, et al., 1999; Cao, et al., 2000; Guo, et al., 2001; Leeson, 2005; Predieri, et al., 2005; Yan and Waldroup, 2006). Certainly, these types of experiments generate useful data, but tissue mineral levels may not be the best marker for mineral bioavailability. First, tissue mineral experiments measure only a fraction of the mineral that is taken up by the animal. Minerals are absorbed by the small intestine, and then distributed via the bloodstream to other tissues. Therefore, tibia or liver mineral levels only measure the mineral that is distributed to those particular tissues, and as such may not reflect total mineral uptake. A second shortcoming is that tissue mineral levels actually represent a storage pool of mineral, rather than the total amount of mineral delivered to that particular tissue. Tibia zinc, for example, measures the amount of zinc that has entered the tibia, less the amount of zinc that has left. Ideally, a true measure of mineral absorption would reflect just the amount of minerals that have been delivered to a particular tissue.

One solution to this problem is to measure the expression of mineral-responsive biomarkers in the animal, preferably in the small intestine where minerals are absorbed. For example, the expression levels of several genes and proteins increase or decrease rapidly depending on the mineral status of the animal. Metallothionein (MT) is such a biomarker, because its expression is regulated by zinc status (Davis and Cousins, 2000). When zinc is absorbed by a cell, it must be bound up quickly into protein, because free zinc can be toxic. The cell therefore responds to zinc uptake by synthesizing MT mRNA (as an intermediate) and then MT protein. The MT protein is then able to bind to the zinc, until it is needed by other enzymes in the cell (McCormick, et al., 1981; Jacob, et al., 1998; Davis and Cousins, 2000). Furthermore, research has shown that in many tissues from a wide variety of species, MT mRNA and protein expression increase when more zinc is taken up, and decrease when less zinc is taken up. As such, MT mRNA or protein expression is widely used as an indicator of the zinc status of humans and animals, and to evaluate the bioavailability of different zinc sources (McCormick, et al., 1981; Lu, et al., 1990; Reeves, 1995; Rojas, et al., 1995; Sullivan, et al., 1998; Blanchard, et al., 2001; Cao, et al., 2002; Martinez, et al., 2004). Novus International, Inc. has developed patent-pending, real-time polymerase chain reaction (RT-PCR) assays to measure the expression of MT mRNA, as a marker for both zinc status and bioavailability. This assay has demonstrated that MT is inducible by zinc in a variety of tissues, including small intestine, liver and kidney (data not shown)

Because OTMs should be more bioavailable than their corresponding inorganic forms, it would be predicted that lower mineral inclusion rates could be used without compromising performance. A commercial broiler trial was conducted to test this hypothesis.

Materials and methods

The bioavailability of three zinc sources was compared to each other and to a zinc-deficient control diet by measuring small intestinal MT as the marker for bioavailability. All birds were fed a milo-soy basal diet for 20 days. The birds were then switched on day 21 to corn-soy treatment diets which consisted of a zinc-deficient control (29ppm Zn from ingredients), or that same control diet formulated to contain an additional 70 ppm Zn from either ZO, ZAAC or MINTREX Zn. MT expression was measured in jejunum mucosal scrapings by RT-PCR two days after the switch to treatment diets, on day 23. Birds were challenged with *E. tenella* (10,000 VSO/bird) on day 30, and tibias were collected on day 35 for zinc analysis by ICP.

In the commercial broiler trial, Cobb 700 broilers (straight run) were fed diets formulated to contain twice the NRC recommended levels of inorganic forms of Zn, Cu and Mn, or the minerals at NRC levels but supplied as a 50:50 blend of inorganic and MINTREX minerals. Growth performance and yield were measured.

Results and discussion

In the bioavailability trial, whereas ZO and the ZAAC only numerically increased MT expression versus the control, animals fed MINTREX Zn expressed significantly greater MT than all other treatments (Figure 1). This result indicates that more zinc from MINTREX Zn than from the other sources was being absorbed by the small intestine. Tibia zinc was also measured in these animals after two weeks on treatment diets. The results from this assay supported the MT results, in that the birds supplemented with MINTREX Zn contained significantly or numerically more tibia zinc than the birds receiving the other diets (data not shown). Together, these results indicate that MINTREX Zn was more bioavailable than the other zinc sources in this experiment.

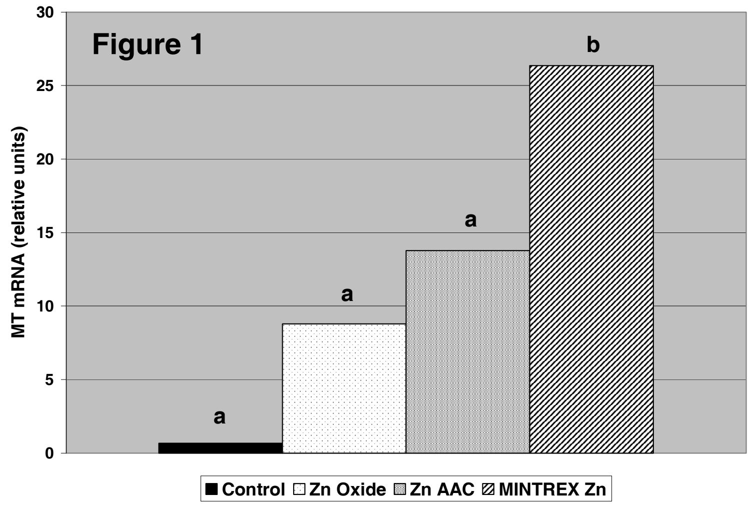

Figure 1 MT expression was greatest in the birds fed MINTREX Zn. MT mRNA expression in broilers fed a control diet unsupplemented for zinc, or the same diet supplemented with 70 ppm zinc from Zn oxide, a Zn amino acid complex, or MINTREX Zn.

Delivering more bioavailable trace minerals should allow one to formulate with lower mineral inclusion, and/or see enhanced benefits in the animal. Indeed, this has been seen with MINTREX. In a commercial broiler trial with straight run Cobb 700 broilers, diets included twice the NRC recommended levels of inorganic forms of Zn, Cu and Mn (NRC, 1994), or the minerals at NRC levels but supplied as a 50:50 blend of inorganic and MINTREX minerals. There were no differences in performance, but the white meat yield in male birds was substantially greater in the ITM/OTM birds at NRC levels than in the 2x NRC ITM birds (22.99% versus 22.38%, respectively). These data indicate that trace mineral inclusions can be reduced greatly if OTMs are used, while at the same time improving yield. Furthermore, by reducing the total Zn, Cu and Mn inclusion by half, mineral excretion would also be expected to be reduced.

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