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KeyShure®

Precision Release Minerals

Balchem Research Summary

Chemical characteristics and relative bioavailability of supplemental organic Zinc sources for poultry and ruminants.

A summary of research conducted at the University of Florida and published as:
J. Cao, P.R. Henry, R. Guo, R.A. Howlerda, J.P. Toth, R.C. Littell, R.D. Miles and C.B. Ammerman; J Anim Sci 2000, Volume 78:2039-2054 and J. Cao, P.R. Henry, S.R. Davis, R.J. Cousins, R.D. Miles, R.C. Littell and C.B. Ammerman; Anim Feed Sci and Tech 2002, Volume 101:161-170.
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Background

Trace minerals are universally recognized nutrients in livestock and human diets for their essential roles in enzyme systems, tissue integrity and immune function. Over the last twenty-five years there has been growing interest, and some evidence, for the use of trace minerals bound to organic ligands due to their hypothesized superior bioavailability in comparison to the inorganic salts of trace minerals. The existing research literature presents inconsistent physiological and production results in animal trials comparing inorganic and organic forms of trace minerals. In addition, there are no standardized chemical methods for the evaluation of organic trace minerals as it relates to the strength or degree of chelation between the trace mineral and its ligand. Lastly, very few studies have correlated chemical characterization with *in vivo* physiological effects. This research bulletin summarizes the results from two papers in which commercially available Zinc (Zn) organic trace minerals were chemically characterized and then compared to inorganic Zn salts in animal trials.

Methods

Chemical Characterization (J Anim Sci, 2000)
Eight commercially available organic trace minerals and reagent grade Zinc sulfate ($ZnSO_4$) were evaluated by several methods to determine: trace mineral and nitrogen content, strength of chelation, solubility at physiological pH, and chelation integrity at physiological pH. The products tested were: two Zn methionine products, a Zn polysaccharide, a Zn lysine, a Zn amino acid (AA) chelate and three Zn proteinates.

Nitrogen and Mineral Content: Samples were dried, ashed, solubilized in HCl, and filtered through ashless filter paper. The Zn content was evaluated by flame atomic absorption spectrophotometry. Nitrogen content was evaluated on a Technicon AutoAnalyzer II.

Polarographic Analysis: Chelation strength was analyzed with a hanging mercury drop electrode to determine the half-wave potential ($\Delta_{E½}$) of saturated solutions of each product. The more positive the $\Delta_{E½}$, the more stable a chelate is.

Solubility at pH 2 and 5: Solubility of each product at concentrations ranging from 0.125 mg/mL up to 12.5 mg/mL were evaluated in buffers at pH 2 and pH 5.

Gel Filtration Chromatography: The soluble fractions from the solubility evaluation were applied to a size exclusion gel and eluted in 0.2 mL aliquots. These were tested for mineral and nitrogen content to determine the separation of free metal ion, amino acids and small peptides, and metal chelates or complexes.

Bioavailability Trials

Experiment 1 (J Anim Sci, 2000)
Four hundred and thirty two broiler chicks were assigned to six pen replicates for each of eight treatments. The basal diet was formulated to meet NRC requirements for Zn. Additional $ZnSO_4$ was added at 0, 200, 400 or 600 mg/kg of DM as one set of treatments or additional Zn was added at either 200 or 400 mg/kg DM from one of two organic trace mineral sources, either a Zn AA chelate or a Zn proteinate (KeyShure Zinc). At 1, 2 and 3 weeks of age, three chicks from each pen were selected and sacrificed. Femurs, intestinal mucosa, intestinal serosa, and liver were harvested for analysis of Zn content or metallothionein activity.

Experiment 2 (J Anim Sci, 2000)
Forty-two cross-bred lambs were assigned to seven treatment groups. The basal diet contained 58 mg/kg of Zn and was formulated to meet NRC requirements for growing lambs. The inorganic Zn treatments included 0, 700, 1400 or 2100 mg/kg of $ZnSO_4$. The organic Zn treatments consisted of 1400 mg/kg of either KeyShure Zinc, Zn AA chelate or Zn methionine. Diets were fed for 21 days, at which time the lambs were sacrificed and samples taken from liver, kidneys and pancreas for analysis of Zn content or metallothionein activity.

Experiment 3 (Anim Feed Sci Tech, 2002)
This experiment differed from Experiment 1 in that supplemented Zn was formulated to be much closer to NRC requirements rather than at pharmacological levels. A secondary objective was to evaluate treatments for a much shorter period of time (9 days) as compared to typical three or six week studies in broilers. Four hundred and thirty two broiler chicks were assigned to six pen replicates for each of eight treatments. The basal diet was formulated to meet or exceed NRC requirements for growing chicks, except for Zn, which was formulated for 24 mg/kg of Zn. The treatments included Zn supplemented at 0, 30, 60 or 90 mg/kg of DM as Zn acetate, or 30 or 60 mg/kg of DM as Zn methionine or Zn proteinate (the same as KeyShure Zinc in the Cao, 2000 paper). At days 3, 6 and 9 of the experiment, three chicks were selected from each pen and sacrificed. Tibias, intestinal mucosa and livers were harvested for analysis of Zn content or metallothionein activity.

Results

Chemical Characterization (J Anim Sci, 2000)
The results of chemical characterization and chelation effectiveness are presented in Table 1. The values in the $\Delta_{E½}$ column represent the voltage required to break the bond between the ligand and trace metal. The higher the value, the stronger the chemical bond. Similarly, the formation quotient, Q_f, is a quantitative measure of chelation effectiveness. Categorically, Q_f values <10 are representative of very weak chelation, values between 10 and 100 represent moderate chelation and over 100 should be considered as strongly chelated.

Zn Source Solubility
There was wide variation in the solubility of the Zn and nitrogen (N) fractions of the commercial products in deionized water as seen in Table 1. The Zn:N ratio for KeyShure Zinc was highly consistent in both soluble and insoluble fractions, indicating that it was the only product which remained chelated under the conditions of this evaluation.

Table 1. Characterization of Chelation Effectiveness of Organic Zn Sources.

Zn Source	$\Delta_{E½}$	Q_f	Zn Soluble*	Zn Insoluble	N Soluble	N Insoluble
Zn Methionine A	.008	1.9	60	5	14	6
Zn Methionine B	.005	1.5	164	3	31	3
Zn Polysaccharide	.017	3.8	348	33	--	7
Zn Lysine	.019	4.4	127	43	66	4
Zn Amino Acid Chel.	.067	180	149	17	48	77
KeyShure® Zinc	.033	13	41	206	45	131
Zn Proteinate B	.058	91	200	26	11	31
Zn Proteinate C	.062	120	216	10	40	58

Key to Chelation Effectiveness (Q_f):

Low	Moderate	High

** All solubility values are reported in mg*

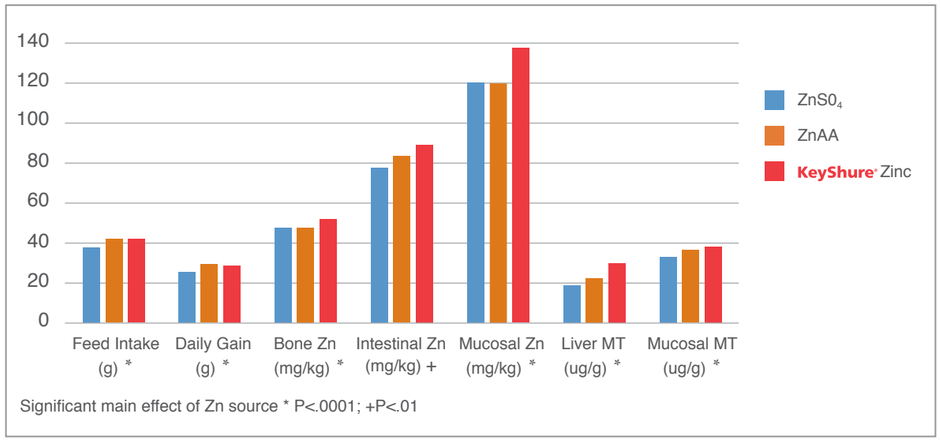
Gel Filtration Chromatography

This method was able to distinguish unique peaks between free Zn ions, amino acids, peptides, complexes and chelates. Applying filtrates of the Zn sources mixed into pH 2.0 and pH 5.0 buffers, to the chromatograph resulted in distinct peaks for free Zn ion, indicating that none of the material remained chelated under these pH conditions. Under conditions of neutral pH, only 2.2% up to 12.2% of the Zn remained bound to its ligand, with the chelates (Zn AA and all Zn proteinates) demonstrating the highest degree of bound Zn (10.2% to 12.2%). The proportion of Zn remaining bound was positively correlated (r=0.96) to the $\log_{10}$ of the chelation effectiveness (Q_f value in *Table 1*).

Experiment 1: Broiler Chick Study (J Anim Sci, 2000)

In this study, only Zn sulfate ($ZnSO_4$), Zn AA chelate and KeyShure Zinc were used as dietary treatments. There were significant main effects of Zn source and supplementation level at each week of the study across all of the response variables of feed intake, daily growth, bone Zn, intestinal Zn, mucosal Zn, liver metallothionein and mucosal metallothionein. The results for the terminal week of the experiment (week 3) and the highest level of Zn supplementation (400 mg/kg of added Zn) are summarized in Figure 1. The estimated bioavailability, based on bone and mucosal Zn, was 104% for Zn AA chelate and 139% for KeyShure Zinc when compared to results with Zn sulfate ($ZnSO_4$).

Figure 1. Effect of Zn Source on Performance and Tissue Zn

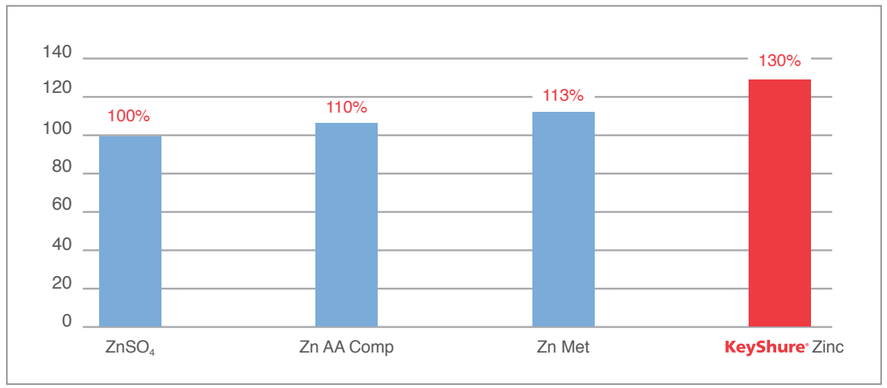


Significant main effect of Zn source * P<.0001; +P<.01

Experiment 2: Lamb Study (J Anim Sci, 2000)

As expected, Zn levels increased in all tissues with increasing Zn intake. When regressing tissue levels on Zn intake, liver Zn had the poorest fit with an R^2 of 0.61. The linear fit for kidney and pancreas Zn and liver metallothionein were 0.73, 0.74 and 0.77, respectively. For these same measures, the slope of the line was greatest for KeyShure Zinc, indicating a higher level of bioavailability than for Zn sulfate ($ZnSO_4$). Based on all four parameters measured, the bioavailability relative toZn sulfate ($ZnSO_4$) (100%) was 110% for Zn amino acid complex, 113% for Zn Methionine B and 130% for KeyShure Zinc (Figure 2).

Figure 2. *Relative Bioavailability of Zinc Sources in Lambs*



Experiment 3: Broiler Chick Study (Anim Feed Sci Tech, 2002)

There were significant effects of age, Zn supplementation level and Zn source on the accumulation of Zn in bone and mucosal metallothionein. Only the Zn proteinate had a significant effect on feed intake (P<.05). The results are summarized in Table 2 for the terminal time point (9 days) and highest Zn supplementation level (60 mg/kg) used across all treatments.

Table 2.

Zn Source	Daily Feed Intake (g)+	Body Weight (g)	Bone Zn (mg/kg)*	Liver MT (ug/g)*	Mucosal MT (ug/g)*
Zn Acetate	19.2	148.7	456	10.5	34.0
KeyShure® Zinc	20.3	163.0	476	14.4	37.0
Zn Methionine	19.3	153.6	436	11.6	33.5

+ Significant main effect of source, P<.05; * Significant main effect of source, P<.001

The mucosal metallothionein response to dietary Zn level was the most consistent response variable for all of the time points with R^2 of 0.83, 0.80 and 0.81 for days 3, 6 and 9, respectively. Using the mucosal metallothionein response at day 9 to calculate bioavailability relative to Zn acetate (100%), Zn methionine was 77% and Zn Proteinate was 130%. In summary, shorter supplementation trials can be effective when Zn is supplemented in ranges closer to animal requirements. Mucosal metallothionein appears to be the most consistent response.

Summary

Across the chemical characterizations and animal trials, KeyShure Zinc demonstrated the most consistent chelation effectiveness and performance. KeyShure Zinc exhibited a moderate chelation strength, consistent solubility of metal and nitrogen components and the highest degree of binding at a neutral pH. In animal trials in broilers and lambs, KeyShure Zinc had the highest bioavailability, >130% relative to $ZnSO_4$, of any of the commercial products tested when assessed by growth performance, and Zn accumulation in bone, tissue or enzyme systems.

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