Feeding Amino Acid Chelated Copper and Zinc to Reduce Mineral Pollution From Swine Manure

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ABSTRACT

High Zn and Cu fecal concentrations impact the environment. Zn and Cu reductions in feed have compromised swine performance. Substituting amino acid chelated (AAC) sources for traditionally employed inorganic salts (IM) may overcome environmental and swine performance problems. A preliminary study demonstrated less ^{65}Zn was deposited in rat feces due to greater AAC absorption compared to IM ($P < 0.01$). This led to a second study using 128 third parity sows from a closed herd divided into 4 equal groups. A supplement containing 27.5 µg/g Cu (from Cu AAC or $CuSO_4$) was given to 2 groups. A second supplement containing 392.9 µg/g Zn (from Zn AAC or $ZnSO_4$) was given to the other groups. Supplementation continued daily for 21 days in normal feed rations. On Day 21, total 24-hour fecal output from each pig was assayed for either Cu or Zn. Pigs fed AAC had significantly less Cu ($P < 0.01$) and Zn ($P < 0.05$) in their feces compared to pigs consuming IM. This demonstrated that supplementing swine rations with Cu and Zn AAC could lower environmental impact through reduced fecal mineral concentrations. Due to greater AAC bioavailability, feed rations could potentially be formulated with lower inclusions of Cu and Zn, without compromising swine's performance.

INTRODUCTION

Depending on body weight, a single pig can produce as much as 6.2 kg of manure per day,[1] which can significantly impact the environment. Traditionally, manure is spread near the swine facility and can potentially create odor, ammonia, and pathogen-carrying dust. It may also lead to soil accumulations of minerals such as P, Cu, and Zn that may pollute ground water or leach into runoff.[2]

Attempts to minimize pollution from manure have fostered alternative swine production methods. These include 1) development of new procedures relating to manure distribution and application, 2) processing the manure and exporting it to deficient soils, 3) reducing fecal production through restricting feed intake, and 4) lowering mineral concentrations in the manure by limiting them in the feed.[3,4]

While these solutions potentially benefit the environment, each presents swine production problems. For example, some point out that growing pigs only utilize 30% to 35% of ingested N and P and conclude that dietary intake of these nutrients should be lowered to meet true requirements.[4] Digestibility is a moving target. It can be modified by the inclusion or exclusion of certain extrinsic enzymes or their co-factors in the feed, by exchanging less digestible feedstuffs for more digestible ones or by modifying the intake of other nutrients that may impact digestion.[5] Further, lowering intake of certain nutrients can potentially affect the health and/or performance of the pig.[3]

Reducing mineral inclusion levels in the feed while maintaining acceptable swine performance is difficult. For example, 5-6 µg/g Cu have been reported to be adequate for growing pigs.[6] However, when 100-250 µg/g Cu, as $CuSO_4$, were added to the feed, early weaned pigs gained 22% faster while consuming 8.3% less feed. When Cu intake was increased from 100 to 250 µg/g in weaner, grower, and finisher feeds, average growth rate during the entire growing period increased 8.9% coupled with a 3.2% reduction in feed intake.[7]

Bowland et al[8] demonstrated that Cu concentration in the feed was not as great a factor on swine growth as the availability of that Cu from the feed. Creech et al[9] reported that when weanling gilts were fed reduced dietary amounts of Cu, Zn, and Mn, with 50% of the reduced amounts sourced from more bioavailable mineral proteinates, gain/feed was greater during the nursery phase compared to pigs receiving the same minerals as sulfates ($P < 0.05$). Lower Zn intake, in the proteinate form, resulted in reduced fecal Zn concentrations ($P < 0.05$).[9] In the grower phase, fecal Cu concentrations were lower in the group receiving Cu proteinate ($P < 0.05$).[9] Based on the above observations, it appears that use of more bioavailable mineral sources in animal feeds may decrease environmental impact from the manure while maintaining animal performance.

MATERIALS AND METHODS

To test this concept, a preliminary trial was contracted for and conducted under a protocol approved by the Institutional Review Board of Weber State University, Ogden, Utah. Forty eight fasted and sedated adult Sprague Dawley male rats of equivalent age and weight were administered 24 µg ^{65}Zn as either an amino acid chelate (AAC) or as $ZnCl_2$. Twenty four animals were assigned to the AAC group, which was further subdivided into 2 groups of 12 each. One AAC group received Zn bisglycinate while the other received Zn bismethioninate. Each 24-µg Zn dosage, from either amino acid ligand source, was dissolved into 200 µL water and each solution administered by gastric gavage. The second group of 24 rats each received 24 µg ^{65}Zn from $ZnCl_2$ in a similar manner. Feces and urine were collected daily from each rat for 7 days.

The feces of each rat were collected on stainless steel screens suspended under the individual cages. The screens allowed the passage of urine through the screens into stainless steel funnels that directed it into glass collection vials. The daily urinary output from each rat was thus recovered and stored in a sealable glass vial. Each day the new urine was added to the previously collected urine so that at the end of the 7-day period, the total urinary output of each rat was mixed together into a single collection vial.

The total 7-day fecal output from each rat was mixed together and dried for 24 hours in an oven at 110°C. Each rat's total fecal output was then ground and blended to create a uniform mixture of the 7-day output. A 0.5-g sample was taken from each blended mixture and ashed in a muffle furnace at 650°C for approximately 8 hours. Once the organic material had burned off, the ash was dissolved in 15 mL of concentrated HCl. When the solution turned clear, it was Q.S. to 25 mL with a 50/50 mixture of a scintillation cocktail and distilled/deionized water.

An aliquot of this solution was placed in a liquid scintillation vial and introduced into a Nuclear Chicago Model 8731 scintillation spectrometer that measured gamma rays with a multi-channel strip recorder. The dissolved fecal ash material from each rat was subsequently analyzed for ^{65}Zn. The data were quenched to provide corrected counts/g dry feces/minute.[10,11]

A 1-mL sample of urine from the 7-day urine collection of each rat was Q.S. to 25 mL with a 50/50 solution of distilled/deionized water and the scintillation cocktail. An aliquot of the solution was placed in a liquid scintillation vial and analyzed for ^{65}Zn using the same Nuclear Chicago Model 8731 scintillation spectrometer that measured ^{65}Zn in the feces.

It was assumed that if the administered ^{65}Zn were not in the feces or urine, it was absorbed and part of the animals' tissues.

The amount of ^{65}Zn in the urine of all animals in all groups was negligible. Consequently, the ^{65}Zn data from the urine was not included in calculating ^{65}Zn retention in the bodies. Once the amount of ^{65}Zn recovered in each rat's feces was determined, the amount of ^{65}Zn retained in each rat's body was calculated. These data were then analyzed for significance using Systat® version 10 (Systat Software, Inc., Richmond CA) and subsequently summarized in Table 1.

Seven days post-dosage, absorption and tissue retentions of Zn from Zn AAC (Zn bisglycinate or Zn bismethionate chelates) were significantly higher compared to Zn from $ZnCl_2$ ($P < 0.01$). The rats administered $^{65}ZnCl_2$ had an apparent absorption of 48% of the ^{65}Zn dose, while 52% was recovered in the feces. Rats receiving ^{65}Zn bisglycinate had an apparent absorption of 88% of the ^{65}Zn dose with 12% being recovered in the feces. Apparent ^{65}Zn absorption from ^{65}Zn bismethionate was 1.9-times greater than that of $^{65}ZnCl_2$.

Based on results of this preliminary ^{65}Zn study, a second trial was designed to determine if pigs fed rations containing Zn or Cu as AAC would also absorb more of these minerals and deposit less into their feces when compared to pigs receiving the same amount of minerals supplied as inorganic mineral salts (IM).

One hundred twenty eight third parity crossbred gestating sows (Landrace × Yorkshire) were selected from a closed herd and divided into 2 groups: Cu (n = 64) and Zn (n = 64). Each group was further subdivided into 2 groups of 32 each. Two of the 4 sub-groups were fed an IM sources of the supplemental minerals (Cu or Zn) while the other two sub-groups received AAC sources of the same minerals. Both AAC and IM supplements provided equal amounts of supplemental Cu or Zn as described below.

Each sow in the study was housed in an individual gestation crate. The gestation crates were 2.03 M long × 0.61 M wide × 0.99 M tall. Water was provided to each sow ad libitum for 23.5 hours each day. Each feed trough was isolated from the crates adjacent to it allowing each pig to be hand fed once daily between 8:00 and 8:30 AM.

The basal ration was formulated to meet NRC requirements for Cu and Zn in gestating sows[6] using Zn oxide (IFN 6-05-553) and Cu sulfate (IFN 6-01-719) as mineral sources.[12] The feed also contained natural intrinsic sources of these minerals in the feedstuffs, which were not part of the NRC calculations. This ration was divided into 4 equal parts and a mineral supplement containing either Cu or Zn was added to each part using AAC (Albion Advanced Nutrition, Clearfield, Utah, USA) or sulfate forms as the mineral sources. The rations were labeled as A, B, C, or D with A corresponding to Cu AAC, B corresponding to $CuSO_4$,

Table 1. Calculated ^{65}Zn Absorption and Measured ^{65}Zn Excretion in Adult Sprague Dawley Rats.

Treatment	N	Amount Zn Administered	Zn Retention in Body	Zn Excretion in Feces	Zn in Urine
^{65}Zn Bisglycinate	12	24 μg	21.1 μg	2.9 μg[a]	Negligible
^{65}Zn Bismethionate	12	24 μg	22.1 μg	1.9 μg[a]	Negligible
^{65}Zn Chloride	24	24 μg	11.5 μg	12.5 μg[b]	Negligible

[a/b]Significant at $P < 0.01$ between AAC and IM treatments.

C corresponding to Zn AAC, and D corresponding to $ZnSO_4$.

The daily feed ration provided to each sow receiving the Cu supplemented feed was 2.170 kg. Supplemental Cu added to the basal feed was formulated to add 27.5 µg/g as either the IM (IFN 6-01-719) or AAC (IFN 6-20-983).[12] Daily feed intake in the Zn group was 2.170 kg. The AAC (IFN 6-20-987) source provided 392.9 µg/g supplemental Zn whereas the IM source (IFN 6-05-555) provided 393.3 µg/g Zn.[12] The quantitative presence of each mineral was confirmed by inductive coupled plasma spectrophotometry (ICP) prior to being fed.[13,14]

Experimental rations containing supplemental Cu or Zn, as either AAC or IM, were fed for 21 days. On Day 21, the 24-hour defecation of each individual sow was collected into plastic containers marked with the sow's individual identification number. Each container was sealed, double-bagged, and sent to an independent analytical laboratory (Servi-Tech Laboratories, Hastings, NE) by air courier where it was dried and Cu and Zn analysis performed.

Cu and Zn concentrations in feces from all sows from each group receiving the AAC sources were compared to Cu and Zn concentrations from feces of sows in each group which received IM sources of Cu and Zn. All individuals handling the pigs, performing laboratory analytical work, or conducting statistical analyses were kept blind as to the source of each mineral.

In this second study, mean concentration values from fecal samples from sows fed either AAC or IM supplemental mineral source were tested for statistical independence using Cochran's t-prime (t') test.[15] The t-values were determined from a cumulative t-distribution chart.[16] For degrees of freedom numbers between 30 and 35, the third places of the t-values for each alpha probability chosen were extrapolated from the integral distance between the t-values for 30 and 35.

RESULTS

The Cu concentration, expressed on a dry weight basis, in the feces from sows receiving supplemental Cu AAC was 271 µg/g ± 25 µg/g, while the value for the sows receiving Cu IM was 310 µg/g ± 39 µg/g. On a mean basis, there was 12.6% less Cu deposited into the feces of sows receiving supplemental Cu AAC as compared to Cu IM ($P < 0.0005$).

The fecal Zn concentration, expressed on a dry weight basis, for sows receiving supplemental Zn AAC was 3770 µg/g ± 319 µg/g, while the fecal Zn concentration for sows receiving Zn IM was 3951 µg/g ± 490 µg/g. On a mean basis, 4.6% less Zn was deposited into the feces of sows receiving supplemental Zn as AAC ($P < 0.05$).

Table 2 summarizes the above results.

Table 2. Concentrations of Zn or Cu in Dried Feces From Sows Fed Amino Acid Chelates or Inorganic Mineral Salt Sources.

Treatment	N	Cu (µg/g)	N	Zn (µg/g)
IM	32	310^{a} ± 39	32	3951^{c} ± 490
AAC	32	271^{b} ± 25	32	3770^{d} ± 319

a/b Significant at $P < 0.0005$ between treatments.

c/d Significant at $P < 0.05$ between treatments.

DISCUSSION

Some investigators have suggested that restricting mineral levels in the feed would alleviate the environmental impact of those metals in the manure.[2,17] If mineral levels were reduced in animals' feeds without regard to the biological roles these minerals play in pigs, it may compromise animal performance including growth rate, feed conversion, or swine health.[5]

If absorption of dietary minerals were increased through feeding AAC sources instead of the traditional IM sources while concurrently lowering mineral inclusion levels in the feed, the net results to both the environment and the pig should be positive. Less minerals would be deposited into feces due to greater absorption of the AAC and lower concentrations of those minerals in the feed would further reduce environmental metal contamination from the manure. Feeding AAC mineral sources would allow optimum pig performance to be maintained because the increased absorption of AAC minerals would compensate for the lower inclusion levels thus providing sufficient minerals for optimum biological needs. While animal performance was not our study objective, we did demonstrate that feeding Cu and Zn AAC to sows resulted in significantly less deposition of those minerals into the feces compared to similar sows fed equivalent amounts of Cu and Zn as IM. This suggests increased uptake of Cu and Zn for enhanced biological purposes in the pigs.

The previous work reported by Kornegay et al[18] indirectly confirms part of this study. They compared Zn absorption from Zn AAC and $ZnSO_4$ in Zn-depleted piglets using chromic oxide as a marker. These investigators recovered approximately 37% more Zn from $ZnSO_4$ than from AAC in the digesta of the small intestine ($P < 0.01$) indicating greater Zn absorption from Zn AAC. There was little difference in Zn recovery in stomach or colon contents between groups. This study also demonstrated that 19% less Zn was deposited in the feces of pigs fed the AAC source of Zn as compared to $ZnSO_4$.

Our ^{65}Zn rat study demonstrated that significantly less ^{65}Zn was recovered in the feces from rats receiving either the glycine or methionine forms of Zn AAC compared to rats receiving IM $ZnCl_2$. The piglet study of Kornegay et al[18] and the preliminary rat study, described above, both exhibited greater Zn absorption than did the pigs in this study. There are several possible reasons for the differences.

The Kornegay piglet study utilized Zn-depleted pigs.[18] When Zn was reintroduced into their diets, both groups probably absorbed more Zn from either source than would have been observed in Zn-sufficient pigs.[19,20] This could affect the magnitude of Zn recovery from the digesta of either group.

The isotope study collected feces daily from rats for the first 7 days following dosing, whereas our swine study only collected a 24-hour sample on Day 21 of the supplementation period. During the preceding 20 days, it is possible that the AAC group of pigs approached Zn saturation and began absorbing less Zn, even though it was in the AAC form. The degree of Zn absorption, from any source, may depend, in part, on Zn plasma and/or tissue levels with an inverse relationship existing between Zn absorption and Zn plasma and/or tissue levels.[19,20] If this inverse relationship came into play with the Zn AAC in our study, then absorption of Zn from the AAC would have been less due to Zn saturation. Zn absorption for the pigs fed Zn IM may not have reached saturation due to its lower bioavailability, and thus, its apparent absorption would have been greater than if these pigs had achieved Zn saturation. The rate of Zn absorption from different supplemental sources needs to be examined in greater detail. Fecal sampling needs to occur more frequently and be initiated earlier in the study in order to obtain a clearer picture.

Lowe et al[21] added another potential dynamic to consider when they reported that intrinsically labeled Zn AAC absorption in dogs was 89.5%. These investigators noted that Zn AAC absorption was directly related to urinary Zn loss. If this relationship exists in pigs, the urinary Zn levels may have contributed to the Zn concentrations measured in the pigs' feces. No attempt was made in our swine study to collect urine. A future study should probably examine this, particularly in view of environmental concerns.

When Zn AAC was administered to test animals, its absorption was consistently greater than Zn absorption from IM sources. When AAC and IM sources were fed in equal amounts, this greater absorption consistently resulted in a significantly lower fecal concentration of Zn. More work needs to be done to determine the magnitude of supplemental mineral reduction that can be obtained under field conditions while maintaining optimum animal production. We suspect that the level of Zn AAC administered in this trial may have been excessive. Dose/response studies as well as balance studies are both indicated.

There are no isotope studies involving Cu to compare with these studies. The short half-life of radioactive Cu precludes practical feeding studies and stable isotopes of Cu AAC have yet to be employed. Nevertheless, the almost 13% less Cu recovered in the feces of sows receiving Cu AAC compared to sows receiving Cu IM suggests greater absorption and retention of Cu from Cu AAC as compared to Cu IM. This agrees with Cu absorption studies in other species of animals.[22,23]

Earlier comments relating to possible tissue Zn saturation resulting from long-term supplementation of Zn AAC[19,20] may also apply to Cu AAC. With greater mineral absorption resulting from feeding Cu AAC, earlier Cu tissue saturation from Cu AAC may have also occurred resulting in reduced Cu absorption during the latter stages of the supplementation period. Consequently the magnitude of comparative difference may change at different supplement intervals. This needs to be investigated more fully in order to establish the optimum supplemental level.

The objective of AAC supplementation is not to achieve tissue saturation but to provide the correct levels of biologically available Cu and Zn needed for optimal animal performance while concurrently lowering fecal concentrations of these supplemented minerals. While studies have reported improved absorption of AAC compared to IM, there are no studies investigating the precise amounts of Cu and Zn AAC required to optimize swine production while simultaneously reducing fecal concentrations of Cu and Zn. This needs to be addressed.

CONCLUSION

Petit and van der Werf[2] have written that the current pig production system is in crisis due to environmental effects of pig manure on water and soil quality. Kirchgessner et al[17] have suggested that in order to reduce the trace element load in soils, considerably lower amounts of supplemental trace element should be added to finisher diets.

This study presents an attractive solution. Including Cu and Zn AAC in swine diets as a portion of the trace element supplementation decreases deposition of those minerals into the feces when compared to mineral concentrations in manure from pigs fed equivalent amounts of the same trace elements as inorganic metal salts. The more efficient absorption of minerals as amino acid chelates compared to minerals from inorganic mineral sources potentially reduces the amounts of supplemental minerals required for optimum swine production. This provides the potential for reducing concentrations of Cu and Zn in swine manure and lowering their impact on the environment while maintaining optimum swine production. Reducing Cu and Zn concentrations in the feed by administering these metals as amino acid chelates should not compromise swine performance. Feed formulas could be developed where most of the supplemented Cu and Zn would be absorbed and utilized by the pig with minimal amounts being deposited into the environment via the manure.

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