Recent Developments in Zinc Bioavailability Research

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INTRODUCTION

Zinc bioavailability estimates in common feed ingredients are very limited (Baker and Ammerman, 1995). Dehulled soybean meal (**SBM**) contains upwards of 60 mg Zn/kg, but there appears to have been only one Zn bioavailability estimate made for this feed ingredient. O'Dell et al. (1972) used either EDTA-treated soy protein or casein and gelatin to construct Zn-deficient diets for chicks. Based on weight gain regressed on the logarithm of supplemental Zn, they concluded that the relative bioavailability (**RBV**) of Zn in SBM (relative to Zn in $ZnCO_3$) was 61% for chicks fed the EDTA-treated soy protein diet and 70% for chicks fed the phytate-free casein-gelatin diet. Rat work on RBV of Zn in soy flours, soy concentrates, and soy isolates has resulted in Zn RBV estimates that range from a low of 23% to a high of 100% (Baker and Ammerman, 1995).

Because EDTA is known to markedly increase Zn bioavailability in phytate-containing feed ingredients (O'Dell et al., 1964), the 61% Zn RBV value for SBM established by O'Dell et al. (1972) may represent an estimate that cannot be applied to phytate-containing corn-soybean meal diets. One of the studies described herein (Edwards and Baker, 2000) was designed to determine RBV of Zn in dehulled SBM, determined in Zn-deficient chicks fed a phytate-containing soy-protein concentrate (**SPC**) diet or a phytate-free egg-white diet.

Considerable confusion exists concerning bioavailability of Zn in various supplements for livestock. Zinc is supplemented in most commercial livestock diets either as $ZnSO_4 \bullet H_2O$ (36% Zn) or ZnO (72% Zn) with Zn from the latter being understood to be less bioavailable than Zn from the former (Wedekind and Baker, 1990). Going further back in the literature, Edwards (1959) reported that Zn from analytical grade (**AG**) and technical grade ZnO as well as Zn metal powder was 100% bioavailable for young chicks relative to AG $ZnSO_4 \bullet 7H_2O$. The following year Roberson and Schaible (1960), also using chick weight gain as the performance parameter, reported that ZnO was as bioavailable as $ZnSO_4$, but they failed to clearly identify either compound. Miller et al. (1981) compared AG ZnO to Zn metal dust in pigs fed a corn-soybean meal diet. They concluded, based on serum Zn data, that the Zn in Zn metal dust was more bioavailable than the Zn in ZnO. Wedekind and Baker (1990) reported RBV values in chicks for Waelz processed ZnO of 61% (weight gain) and 44% (total tibia Zn) when compared to feedgrade (**FG**) $ZnSO_4 \bullet H_2O$ (100%). Wedekind et al. (1994) concluded, based on bone Zn accumulation in pigs, that FG ZnO was only about 68% as bioavailable as FG $ZnSO_4 \bullet H_2O$. Edwards et al. (1998) evaluated two byproducts of the galvanizing industry, Fe-$ZnSO_4 \bullet H_2O$ and Zn-$FeSO_4 \bullet H_2O$ and found both to be as bioavailable, based on weight gain and total tibia Zn, as FG $ZnSO_4 \bullet H_2O$.

There has not been a thorough evaluation of Zn bioavailability for the principle Zn sulfate monohydrates and Zn oxides used by the feed industry today. Using AG zinc sulfate heptahydrate as the standard, the bioassays herein were designed to establish Zn RBV estimates for the following compounds (Table 1): foodgrade $ZnSO_4 \bullet H_2O$ (white powder); a FG $ZnSO_4 \bullet H_2O$ (FG-1, white powder) produced domestically; a FG $ZnSO_4 \bullet H_2O$ (FG-2), white powder) produced in Mexico; analytical grade ZnO (white powder); a FG ZnO (FG-1, pale yellow powder) manufactured domestically by the hydrosulfide process; a FG ZnO (FG-2, greenish-brown powder); manufactured in Mexico by the Waelz process; a FG ZnO (FG-3, fine black granules) manufactured in China (process unknown); a feedgrade ZnO (FG-4, grayish-white powder) manufactured in Mexico by the French process; Zn metal dust (100% Zn, black powder); and a feedgrade Zn metal fume (also known as KO61, reddish-brown powder).

Table 1: Characterization and relative bioavailability of zinc sources investigated[a]

Zn source	Zn level, %[c]	Zn RBV, %[d]	Color	Manufacturing process
$ZnSO_4 \bullet 7H_2O$	22.7	100	White	Unknown (U.S.)
ZnO (FG-1)	78.1	95	Pale yellow	Hydrosulfide process (U.S.)
ZnO (FG-2)	74.1	37	Greenish-brown	Waelz process (Mexico)
ZnO (FG-3)	69.4	47	Charcoal	Unknown (China)
ZnO (FG-4)	78.0	84	Grayish-white	French process (Mexico)
ZnO (AG)	80.3	89	White	Unknown (U.S.)
$ZnSO_4 \bullet H_2O$ (FG-1)	36.5	86	White	Unknown (U.S.)
$ZnSO_4 \bullet H_2O$ (FG-2)	35.3	87	White	Unknown (Mexico)
$ZnSO_4 \bullet H_2O$ (Foodgrade)	36.5	89	White	Unknown (U.S.)
Zn metal dust	100.0	67	Black	Unknown (U.S.)
Zn metal fume	91.5	36	Reddish-brown	Unknown (U.S.)

[a] Appreciation is expressed to Jon Nelson, Southeastern Minerals, Corp., Bainbridge, GA for assistance in obtaining and characterizing the feedgrade Zn products used.
[b] AG = analytical grade; FG = feedgrade.
[c] Determined by atomic absorption spectrophotometry following wet ashing.
[d] Relative bioavailability (RBV) was calculated from the standard curve regressions, setting RBV of Zn in the $ZnSO_4 \bullet 7H_2O$ standard at 100%.

METHODS

General Procedures

Housing, handling, feeding, and killing procedures were in accord with protocols approved by the University of Illinois Committee on Laboratory Animal Care. In all trials New Hampshire x Columbian male or female chicks were fed a standard 23% crude protein corn-soybean meal diet from d 0 to d 3 post-hatching, after which they were fed a Zn-deficient SPC diet (Table 2) until 1600 h on d 7 post-hatching. On d 8 following 16 h of feed deprivation, chicks were individually weighed, wing banded and randomly allotted to pens such that each pen had a similar initial weight and weight distribution.

Four replicate groups of four chicks were allowed *ad libitum* access to the experimental diets and deionized water for 12 d of feeding. Chicks were maintained in heated thermostatically controlled stainless steel batteries (Petersime Incubator Co. Gettysburg, OH) with raised wire floors. To minimize Zn contamination from the environment, stainless steel feeders and waterers were also used. Chicks were exposed to constant fluorescent light 24 h per day.

The two basal diets (Table 2) were formulated to contain 20.1% CP and 1.0% Ca, and they were adequate to superadequate in all nutrients except Zn (NRC, 1994), which was present at 13.5 and 0.3 mg Zn/kg (as fed) for the SPC and egg white diet, respectively (atomic absorption analysis). The NRC (1994) requirement for young chicks fed corn-SBM diets is 40 mg/kg. Because commercial egg white is high in salt, the mineral salt mix used for the SPC diet was not used for the egg white diet. Instead, individual mineral salts were used to meet mineral requirements for the egg white diet. Also, extra biotin was added to the egg white diet to guard against the possibility that residual avidin might have been present. Ingredient additions were made to basal diets in place of cornstarch. Upon termination of each experiment, chicks and feeders were weighed, and chicks were then killed by CO_2 gas. Previous research in our laboratory had shown that chicks pretested as done herein and then fed a Zn-deficient SPC diet similar to that shown in Table 2 would be expected to show linear increases in weight gain when $ZnSO_4 \bullet 7H_2O$ is supplemented at levels between 0 and 10 mg/kg (Wedekind and Baker, 1990; Wedekind et al., 1992; Edwards et al., 1998).

Table 2: Composition of soy concentrate and egg white basal diets (as fed basis)

	Percent (or mg/kg)	
Ingredient	Soy concentrate diet[a]	Egg white diet[a]
Cornstarch/Dextrose	-	to 100
Cornstarch	to 100	15.89
Dextrose	36.92	15.89
Soy concentrate	31.00	-
Egg white	-	25.00
Soybean oil	5.00	5.00
Salt mix (Zn free)[b]	5.37	-
Solka floc	-	3.85
Tricalcium phosphate	-	2.40
DL-methionine	0.20	-
L-threonine	0.10	-
L-arginine	-	0.10
Vitamin premix[c]	0.20	0.20
Biotin	-	60 mg/kg
Choline chloride	0.20	0.20
$MgSO_4\bullet7H_2O$	-	0.35
$KHCO_3$	-	0.20
$MnSO_4\bullet H_2O$	-	231 mg/kg
$FeSO_4\bullet7H_2O$	-	375 mg/kg
$CuSO_4\bullet5H_2O$	-	20 mg/kg
KI	-	0.15 mg/kg
Na_2SeO_3	-	0.22 mg/kg
DL-α-tocopheryl acetate	20 mg/kg	20 mg/kg
Ethoxyquin	125 mg/kg	125 mg/kg

[a] Additions to the basal diets were made at the expense of cornstarch. Analyzed Zn concentrations were 13.5 mg Zn/kg for the SPC diet and 0.3 mg Zn/kg for the egg white diet.
[b] Provided (per kilogram of diet): $Ca_3(PO_4)_2$, 28.0 g; K_2HPO_4, 9.0 g; NaCl, 8.89 g; $MgSO_4\bullet H_2O$, 3.5 g; $CaCO_3$, 3.0 g; $MnSO_4\bullet H_2O$, 0.65 g; $FeSO_4\bullet7H_2O$, 0.42 g; KI, 40 mg; $CuSO_4\bullet5H_2O$, 20 mg; $Na_2MoO_4\bullet2H_2O$, 9 mg; H_3BO_3, 9mg; $CoSO_4\bullet7H_2O$, 0.1 mg; Na_2SeO_3, 0.22 mg.
[c] Provided (per kilogram of diet): thiamin•HCl, 20 mg; niacin, 50 mg; riboflavin, 10 mg; D-Ca-pantothenate, 30 mg; vitamin B_{12}, 0.04 mg; pyridoxine•HCL, 6 mg; D-biotin, 0.6 mg; folic acid, 4 mg; menadione dimethylpyridinol bisulfate, 2 mg; ascorbic acid, 250 mg; cholecalciferol, 15 μg; retinyl acetate, 1789 μg.

RESULTS AND DISCUSSION

Table 3 illustrates how vastly different Zn RBV values can be obtained, depending on how utilization of the inorganic Zn standard is affected by the presence or absence of Zn antagonizing factors, primarily phytate, in the basal diet. In effect, the phytate present in the SPC affected Zn utilization more when $ZnSO_4\bullet7H_2O$ was supplemented than when SBM was supplemented. This lowered the slope of the standard curve, which on a relative basis effectively increased the Zn RBV of SBM to 78% as compared to the 40% Zn RBV value estimated for SBM using the egg white diet (Figure 1). It appeared, in fact, that slope of the linear regression line resulting from feeding the egg white diet (51.2 g gain per mg of supplemental Zn intake) was decreasing between the second and third Zn dosing point. The linear regression equation for the egg white diet was $Y = 87.9X + 52.0$ when using only the first two dosing points (0 and 4.03 mg Zn/kg from $ZnSO_4\bullet7H_2O$). Using this equation to estimate the RBV of SBM resulted in a Zn RBV value of 34 ± 1.3%.

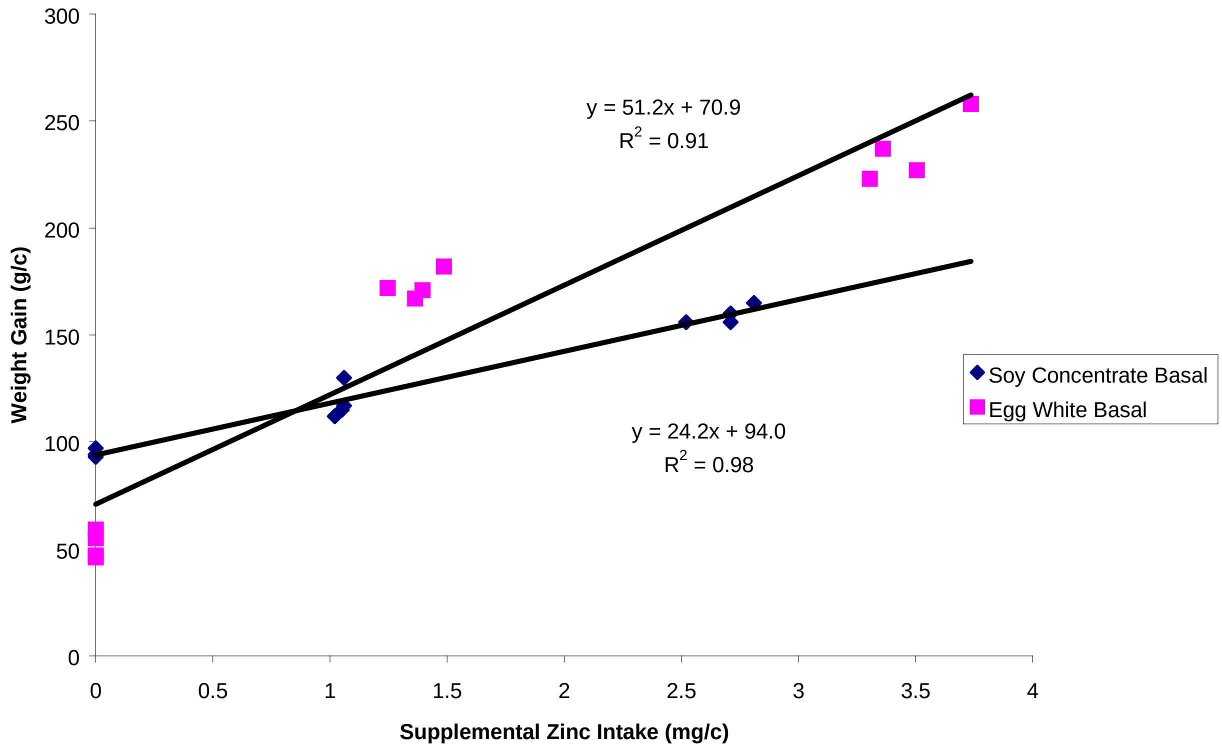

Figure 1: Linear regression plots of the first three doses of Zn from $ZnSO_4 \bullet 7H_2O$ for the SPC diet and the egg white diet. Data were taken from Table 3, and each data point represents the mean of four pens of four chicks.

Feed formulation in practice involves a decision as to what Zn RBV value is appropriate for the SBM contained in a corn-SBM diet. We believe the 78% RBV value is a more accurate representation than the 40% RBV value obtained herein using an egg white basal diet. Whereas the 78% Zn RBV value is lower than the Zn RBV value for AG $ZnSO_4 \bullet 7H_2O$, it is actually higher than the Zn RBV value of 34% estimated for Waelz-processed (FG) ZnO, the Zn supplement used most widely in livestock diets (Edwards and Baker, 1999).

With as much as 40% SBM contained in most broiler and turkey diets, it could be questioned whether supplemental inorganic Zn is necessary in these diets. Thus, the work herein supports the view that the Zn in SBM is much better utilized than most of us had assumed.

The most striking finding in the assays in which various inorganic FG Zn sources were evaluated was the wide range of RBV values for the ZnO products. This has implications for both the feed industry and the animal production industry, not only because of cost per unit of bioavailable Zn, but also because unabsorbed Zn could contribute to environmental concerns about soil buildup of Zn. Feed manufacturers prefer to use oxide salts of trace minerals, including Zn, because oxide salts are less reactive and they contain up to twice the cation concentration as sulfate salts. They therefore occupy less *space* in trace-mineral premixes.

Table 3: Zinc bioavailability in soybean meal using zinc deficient soy concentrate[a] and egg white[b] diets[c]

Diet	Supplemental Zn intake (mg)	Weight gain[e] (g)	Gain:feed (g/kg)
1. Soy concentrate basal diet	0[i]	95[i]	383[h]
2. As 1 + 4.06 mg Zn/kg[d]	1.05[h]	119[h]	460[g]
3. As 1 + 8.39 mg Zn/kg[d]	2.69[g]	159[g]	498[g]
4. As 1 + 50.88 mg Zn/kg[d]	20.78[f]	236[f]	578[f]
5. As 1 + 15% SBM (9.89 mg Zn/kg)	3.18[g]	154[g]	484[g]
Pooled SEM[k]	0.28	6	23
6. Egg white basal diet	0[j]	52[i]	324[i]
7. As 1 + 4.03 mg Zn/kg[d]	1.37[i]	173[g]	509[g]
8. As 1 + 8.00 mg Zn/kg[d]	3.48[g]	236[f]	544[g]
9. As 1 + 50.03 mg Zn/kg[d]	20.16[f]	246[f]	609[f]
10. As 1 + 10.9% SBM (7.19 mg Zn/kg)	2.09[h]	114[h]	397[h]
Pooled SEM[k]	0.23	8	19

[a] Data are means of four pens of four male chicks fed the experimental diets from d 8 to d 20 post-hatching; average initial weight was 96 g.
[b] Data are means of four pens of four female chicks fed the experimental diets from d 8 to d 20 post-hatching; average initial weight was 88 g.
[c] Chicks were pretested from d 0 to d 3 on a 23% CP fully fortified corn-SBM diet, after which they were switched to a Zn-deficient soy concentrate diet from d 3 to d 7 post-hatching.
[d] From analytical grade $ZnSO_4{\bullet}7H_2O$.
[e] Weight gain regressed on supplemental Zn intake for diets 1 to 3 produced the equation $Y = 94.0 + 24.2\ X$ ($r^2 = 0.98$). Standard-curve methodology resulted in a predicted relative Zn bioavailability in SBM of 78 ± 1.5%. Weight gain regressed on supplemental Zn intake for diets 6 to 8 produced the equation $Y = 70.9 + 51.2\ X$ ($r^2 = 0.91$). Standard-curve methodology resulted in a predicted relative Zn bioavailability in SBM of 40 ± 5%.
[f-j] Means in columns with unlike superscript letters are different ($P < 0.05$)
[k] Linear ($P < 0.01$) response to supplemental Zn (diets 1 to 3 and diets 6 to 8) for all response criteria.

Not surprising, analytical grade ZnO was found to be as efficacious (89% RBV) as the analytical grade $ZnSO_4{\bullet}7H_2O$ standard, which agrees with the results reported by Edwards (1959). Among the FG sources of ZnO the process by which it was manufactured had a great effect on the measured RBV value. Feedgrade ZnO, manufactured by the hydrosulfide process and examined in all three assays (94%, 97%, 93% RBV based on weight gain) distinguished itself as a consistent performer that was not different from the $ZnSO_4{\bullet}7H_2O$ standard. ZnO FG-4, manufactured by the French Process, also performed well (84% RBV) relative to the standard. The Waelz product (ZnO FG-2), also examined in all three assays, performed poorly relative to the standard (32%, 41%, and 39% RBV based on weight gain). These results corroborate the results of Wedekind and Baker (1990) who reported RBV values from 44 to 61% for Waelz-processed ZnO relative to FG $ZnSO_4{\bullet}H_2O$. The work herein suggests that had Wedekind and Baker (1990) used a $ZnSO_4{\bullet}7H_2O$ standard, their RBV estimates would have been similar to those reported herein. Feedgrade ZnO's from China can literally come from hundreds of sources (J. Nelson, personal communication, 1999). Knowing how the product has been processed can be problematic. The product we evaluated (ZnO FG-3) was similar in appearance to the Waelz-processed ZnO product, but was blacker and more granular; it also performed somewhat better than the Waelz-processed ZnO.

There was very little difference among the FG zinc sulfate monohydrates. All were similar in appearance, and RBV values were virtually the same (89% foodgrade, 86% FG-1, and 87% FG-2) and not different from the standard. The 67% RBV estimate for 100% Zn metal dust is lower than that reported by Edwards (1959). A possible explanation for this discrepancy is that although Edwards (1959) used the same standard ($ZnSO_4{\bullet}7H_2O$) that we did, his standard curve was derived using additions of 0, 10, and 20 mg Zn/kg whereas we used additions of 0, 5, and 10 mg Zn/kg. Our experience suggests, when using weight gain as the performance parameter, the standard curve begins to plateau at close to 10 mg/kg of supplemental Zn when added to a soy concentrate diet, which Edwards (1959) also used. We suspect his estimate was high because the upper dosing points of his standard curve would have had the effect of lowering the slope of the standard curve.

The Zn metal fume product (KO61) generated a poor RBV value (36%) and this was not unexpected. Horstmeier (1998) discussed the fact that this product has a low solubility and a high iron content.

CONCLUSIONS

Zinc-antagonizing components, primarily phytate, in soy products reduce the utilization of inorganic Zn added to soy containing diets. Hence, when a Zn-deficient soy concentrate diet is used to assess Zn bioavailability in SBM relative to a standard such as $ZnSO_4{\bullet}7H_2O$, a relatively high bioavailability value of 78% is obtained. This value is more realistic for use in practical feed formulation than the 40% value obtained when a phytate-free egg white diet is used to assess the relative bioavailability of Zn.

Feedgrade sources of ZnO are variable in appearance (color, texture), zinc content, processing method, and relative bioavailability values. The same cannot be said for the FG sources of $ZnSO_4{\bullet}H_2O$ whose appearance, zinc content, and bioavailability values are virtually the same. End users of these products in the feed industry should consider not only the physical properties (flowability, storage, stability) but also the cost per unit of bioavailable Zn.

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