Review

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Interactions of zinc with phytate and phytase in the digestive tract of poultry and pigs: a review

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Abstract

Phytase supplementation is gaining importance in animal nutrition because of its effect on phosphorus (P) digestibility and the increasing relevance of P for sustainable production. The potential inhibitors of phytase efficacy and phytate degradation, such as calcium (Ca) and zinc (Zn), have been a subject of intense research. This review focuses on the interactions of Zn with phytate and phytase in the digestive tract of poultry and pigs, with an emphasis on the effects of Zn supplementation on phytase efficacy and P digestibility. *In vitro* studies have shown the inhibitory effect of Zn on phytase efficacy. However, relevant *in vivo* studies are scarce and do not show consistent results for poultry and pigs. The results could be influenced by different factors, such as diet composition, amount of Zn supplement, mineral concentrations, and phytase supplementation, which limit the comparability of studies. The chosen response criteria to measure phytase efficacy, which is mainly tibia ash, could also influence the results. Compared to poultry, the literature findings are somewhat more conclusive in pigs, where pharmacological Zn doses (≥ 1000 mg kg^{-1} Zn) appear to reduce P digestibility. To appropriately evaluate the effects of non-pharmacological Zn doses, further studies are needed that provide comprehensive information on their experimental setup and include measurements of gastrointestinal phytate degradation to better understand the mechanisms associated with Zn and phytase supplements.

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Keywords: phytate; phytase; zinc; non-ruminants; inositol phosphate

INTRODUCTION

Phosphorus (P) is an essential element in animal nutrition, and its adequate supply is important to maintain animal health and performance. However, P from plant seeds and feedstuffs produced thereof is only available to non-ruminants to a limited extent[1,2] because it is mainly present as phytic acid (*myo*-inositol-1,2,3,4,5,6-hexakisphosphate; $InsP_6$) or phytate, which is the salt form of phytic acid. To reduce P excretion of animals and ensure saving finite global rock P deposits by avoiding the use of feed phosphates, commercial feed for pigs and poultry is widely supplemented with phytases. Commercially used phytases belong to the group of histidine phosphatases, which have a conserved catalytic core with histidine in the center.[3] Phytases improve P digestibility by cleaving P from $InsP_6$.[1] Therefore, it is crucial to determine the potential inhibitory factors of $InsP_6$ degradation in the digestive tract of pigs and poultry.

In plant feedstuffs, the native zinc (Zn) content is generally low, and its availability to non-ruminant animals is limited and variable because of the presence of dietary antagonists.[4] Therefore, supplementing plant-based diets with exogenous Zn sources to fulfill the demands of Zn in non-ruminants is a common practice. Inorganic Zn sources, such as $ZnSO_4$ or ZnO, or chelated Zn sources are used as exogenous Zn sources. $InsP_6$ has been reported as a potential complexing agent for exogenous Zn and other divalent cations such as copper (Cu) and calcium (Ca) since the 1960s[5] and the affinity of Zn to $InsP_6$ in particular is very high.[5] *In vitro* studies have shown a decreased P release by phytase in an $InsP_6$ solution in the presence of Zn.[6] A high $InsP_6$ concentration in the diet is associated with

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reduced Zn availability;[7] however, whether the native Zn is already bound to $InsP_6$ in the plant is controversial.[8] *In vivo* studies have produced inconsistent results on interactions of Zn and $InsP_6$. Reduced Zn bioavailability in pigs and poultry in the presence of $InsP_6$ has been reported in single studies.[9,10] By contrast, a meta-analysis of studies revealed that supplemental inorganic Zn and dietary $InsP_6$ did not interact with each other because supplementation of inorganic Zn increased the bone Zn concentration in broilers and piglets regardless of the $InsP_6$-P concentration in the diets of the animals.[11] Therefore, it is necessary to take a closer look on this topic and investigate potential interactions between Zn and $InsP_6$ as these might have implications for feed formulations.

Some reviews have focused on the effect of phytase on Zn bioavailability.[12,13] To our knowledge, the reverse effect (i.e. the effect of Zn supplementation on phytase efficacy) has not been reviewed till date. Therefore, the present review aims to provide an overview of *in vitro* and *in vivo* studies that have investigated the interactions of Zn with $InsP_6$ and phytase, with particular emphasis on the potential effect of Zn supplementation on phytase efficacy.

EFFECTS OF ZN ON PHOSPHORUS-RELATED TRAITS

Interactions between Zn and $InsP_6$, phytase, or both are essential for affecting P-related traits by Zn. Such interactions require specific conditions, as demonstrated in *in vitro* studies. Both Zn and $InsP_6$ must be present in their ionic forms, and the pH of the surrounding environment and the molar ratio of Zn to $InsP_6$ play a crucial role.[6,14] Therefore, the probability of Zn^{2+} encountering an ionized $InsP_6$ molecule *in vivo*, resulting in interactions, varies depending on its localization in the digestive tract. The *de novo* formation of insoluble Zn-$InsP_6$ complexes is most likely to occur in the small intestine because of the higher intestinal pH compared to the gastric pH. However, most exogenous phytases have an optimum pH in the range of 3.0–5.5, which prevails in the proximal digestive tract, and a large part of $InsP_6$ is already degraded in the stomach, making Zn-$InsP_6$ complex formation less likely to occur in the small intestine.[15,16]

Differences in the results of *in vitro* and *in vivo* studies as well as among *in vivo* studies are expected. Different $InsP_6$ sources exhibit different susceptibilities towards cations. Native $InsP_6$ in different plant feedstuffs and pure sodium (Na)-$InsP_6$ (usually used *in vitro*) differ in the rate of hydrolysis by phytase because of the differences in solubility and accessibility.[17,18] Less significant differences in the solubility behavior of corn and wheat $InsP_6$ were found,[19] whereas a significantly higher proportion of $InsP_6$ from soybean meal was hydrolyzed compared to $InsP_6$ from rapeseed meal.[20,21] Therefore, the composition of the diet should be considered when interpreting the study results. Furthermore, in many *in vivo* studies, the $InsP_6$-P concentration in the diet was simply calculated using table values and was not analyzed. The $InsP_6$-P concentration can differ depending on grain variety, climatic conditions, location, irrigation conditions, soil type, and feed ingredient processing; therefore, table values may not be useful in the context of such work.[22,23] To date, in publications, the lack of analyzed $InsP_6$ concentrations in the feed has made it impossible to evaluate the effect of different $InsP_6$ concentrations. It is essential to analyze the $InsP_6$ concentrations of the study material in future studies. It should be noted, however, that, depending on the method used for $InsP_6$ analysis, analysis of individual ingredients may be preferable to analysis of complete feed. For example, the 'ferric chloride precipitation' method, which is a standard method for $InsP_6$ analysis, is not suitable for analysis of $InsP_6$ in complete feed.[24]

Poultry

Tibia ash is a widely used trait for evaluating Zn and P supplementation effects and phytase efficacy *in vivo*.[25] The addition of 32 mg kg^{-1} Zn from a Zn-amino-acid complex to a Zn-deficient diet together with phytase supplementation caused a significant increase in the amount of tibia ash compared to the diet with phytase supplementation alone.[26] Another study, where Zn supply was sufficient in all treatments, found a significant reduction in tibia ash after high ZnCl and phytase supplementation compared to phytase supplementation alone (Table 1).[27] In the latter study, it was assumed that Zn inhibited $InsP_6$ degradation by phytase because of the reduced accessibility of the $InsP_6$ molecule as a result of complex formation with Zn (Fig. 1). In ducks, supplementation of ZnO together with phytase to a non-Zn-supplemented corn-soybean meal-based diet caused a decrease in tibia ash concentration compared to phytase supplementation alone.[28] Because the effect of added Zn on tibia ash in the latter two studies occurred only in combination with phytase supplementation, a direct inhibition effect of Zn on phytase can be suspected (Fig. 1).[29] This appears to be plausible because Zn^{2+} is known to be an inhibitor of many enzymes, in that it is very reactive and may bind to the active site of enzymes. It could be speculated that Zn^{2+} might interact with the side chain of histidine in the catalytic core because Zn^{2+} ions can potentially interact strongly with the side chain of histidine.[29] However, this is only a speculation because, to our knowledge, there is no information available on how Zn^{2+} binds to phytase.

In vitro studies and few *in vivo* studies showed a clear effect of Zn supplementation on phosphate release from $InsP_6$[6,14] or tibia ash concentration.[27,28] However, no effect of Zn supplementation on tibia ash concentration was observed in most *in vivo* studies, whether phytase was supplemented or not.[11,30,31] Additionally, the interaction between the Zn supplementation level and phytase supplementation was not significant in most studies. The effects of Zn on P-related traits were divergent (Table 1), indicating the inconsistent results on the effects of Zn reported in various studies. Differences in the age of the birds and duration of the experimental feeding probably contributed to such inconsistencies.

Differences in the statistical significance of treatment effects among studies may depend on whether tibia ash is expressed as concentration or total amount because concentration and amount do not consistently respond to the same magnitude.[26] In assessing bone mineralization, the amount of tibia ash has been shown to be more accurate than the ash concentration.[32] Quantities of ash appear to be more sensitive to breed, age, and feed intake than ash concentrations. Furthermore, less sensitive results were reported in a 10-day bioassay of tibia ash compared to a 4-week bioassay; therefore, the duration of experimental feeding must also be taken into account.[33] Furthermore, phytase supplementation to the feed may not show effects on tibia ash if the Ca and P requirements of the animals are fulfilled. Other P-related traits are less dependent on the P requirement of the bird in the response to P addition. For example, prececal P digestibility was shown to have a linear response to P intake over the tested range of P (2.5–8.0 g kg^{-1}) in the feed.[34] This indicates that the determination of prececal $InsP_6$ degradation may be a more suitable way to evaluate Zn effects on phytase efficacy.[35] The prececal $InsP_6$ degradation can be used to assess the digestibility of

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Table 1. Zn effects on phytase efficacy as determined by tibia ash in poultry

Study	Experimental feeding (duration in days)	Native Zn (mg kg^{-1})	Targeted Zn supplement. (mg kg^{-1})	Exogenous Zn source	Targeted phytase levels (FTU kg^{-1})	Phytase origin	Phytate-P (g kg^{-1})	Phosphorus (g kg^{-1})	Calcium (g kg^{-1})	Zn effect on tibia ash		
											Unit	Phytase × Zn
(11)	2–21	38	0, 15	$ZnSO_4$, ZnGly	0, 500	*Aspergillus niger*[b]	2.3	10.4	14.0	NS	g kg^{-1} DM	NS
(26)	1–21	25	0, 2, 4, 8, 16, 32	ZnPro	0, 500	i.m.	≈ 2.2	aP = 4.5	10.0$^{(cal.)}$	↑	mg	[e]
(27)	8–20	≈ 33	75 (+800)	ZnO, (+$ZnCl_2$)	0, 500	*Escherichia coli*[c]	≈ 2.2	4.2	7.5$^{(cal.)}$	↓[a]	%	[e]
(28)	1–56	26	0, 30	ZnO	0, 500	*Escherichia coli*[d]	S ≈ 2.3, F ≈ 2.1	aP: S = 4.5, F = 4.3	S = 9.5, F = 9.2$^{(cal.)}$	↓[a]	%	[e]
(30)	1–35	S = 26, F = 23	0, 15	$ZnSO_4$	0, 500	i.m.	S ≈ 2.3, F ≈ 2.1	aP: S = 5.0, F = 4.5	S = 9.9, F = 9.2	NS	%	NS
(31)	5–21	37	0, 14, 35	$ZnSO_4$	0, 800	*Aspergillus niger*[b]	2.4$^{(cal.)}$	aP = 4.3	9.8$^{(cal.)}$	NS	g	NS
(31)	5–21	31	14, 35	$ZnSO_4$	0, 800	*Aspergillus niger*[b]	3.1$^{(cal.)}$	aP = 4.2	≈ 5.2	↑[a]	g	[e]
(31)	5–21	34	0, 10, 30	$ZnSO_4$	0, 1200	*Aspergillus niger*[b]	1.9$^{(cal.)}$	aP = 4.3	10.1$^{(cal.)}$	NS	g	NS
(31)	5–21	22	10, 30	$ZnSO_4$	110, 480	*vegetal*	2.0$^{(cal.)}$	aP = 4.6	≈ 10.8	NS	g	NS

Note: Phytase × Zn, interaction of phytase and Zn; NS, not significant; cal., calculated; ≈, subsequently calculated with table values; ZnGly, Zn glycine chelate; ZnPro, Zn proteinate chelate; S, Starter; F, Finisher; i.m., information missing; aP, *calculated* 'available' phosphorus.
[a] Only in presence of exogenous phytase.
[b] Natuphos.
[c] EcoPhos.
[d] 6-phytase, Danisco Animal Nutrition.
[e] Significant interaction.

$InsP_6$-P and to investigate the complex formation of $InsP_6$ with Zn. Tibia ash is responsive to the overall supply of digestible P. Once absorbed, it is not possible to distinguish whether the phosphate has originated from $InsP_x$ (all inositol phosphates, disregarding the degree of phosphorylation) or other P sources; therefore, bone ash is not a good indicator for evaluating the effects of phytase. However, $InsP_x$ (and *myo*-inositol) in the distal part of the ileum can be measured to determine the amount of P released from the $InsP_6$.[2] Yet, implementing $InsP_x$ analysis on a broader scale may be difficult because $InsP_x$ analysis by chromatography is expensive and assays applicable in commercial laboratories seem not to be developed yet.

Exogenous Zn sources can differ greatly in their chemical characteristics, such as solubility and reactivity. These chemical characteristics affect their passage through the digestive tract (Fig. 1) and may have varying effects on phytase efficacy.[14] However, supplementation with $ZnSO_4$ had no effect on tibia ash in several studies (Table 1). In addition to Zn, supplementation of other trace elements contributes to the presence of interacting cations in the digestive tract. This may further complicate the interpretation of the results as the level of supplementation varies among studies. For example, Cu supplementation in the studies listed in Table 1 ranged from 4 to 20 mg kg^{-1} and Fe supplementation ranged from 20 to 80 mg kg^{-1}. Limestone and ZnO may contain significant amounts of Fe_2O_3, which are not reported by default.[36] Because it was proposed that Fe might affect phytase efficacy,[37] this could lead to bias when Fe-containing Zn supplements are used.

The Ca of the feed may also be a confounding factor, since high levels of Ca, especially in the form of $CaCO_3$, can increase the pH in the digestive tract,[38] making Ca an additional variable for Zn-$InsP_6$ interactions. It was hypothesized that Ca might enhance the negative effect of $InsP_6$ on Zn bioavailability, and a higher ratio of $InsP_6$ × Ca:Zn might indicate poorer Zn bioavailability because of the increased formation of insoluble Zn-Ca-$InsP_6$ complexes in the small intestine.[39] It was observed that high levels of Ca further decreased Zn availability in the presence of $InsP_6$.[40] With an increasing presence of Ca, the number of occupied binding sites of $InsP_6$ molecules increases, increasing the precipitation probability of the complex because the complex solubility decreases with increasing number of occupied binding sites. However, other studies questioned the higher predictive value of the $InsP_6$ × Ca:Zn ratio for Zn bioavailability than Zn:$InsP_6$, hypothesizing that Ca might compete with Zn for $InsP_6$ binding sites.[41]

Another factor that might have contributed to the low comparability among various studies is the origin of phytase. Phytases produced by different evolutionary distant microorganisms, such as bacteria and fungi, consist of fundamentally different proteins with different chemical characteristics.[16] In an *in vitro* study, the efficacy of *Escherichia coli* phytase was reduced to 20% of the control after Zn supplementation (25 ppm Zn from $ZnSO_4$), whereas the efficacy of *Aspergillus niger* phytase decreased only to 70% of the control.[14] Consistently, the results of *in vivo* studies showed that Zn supplementation to diets containing an *E. coli* phytase may decrease tibia ash; however, Zn supplementation to a diet containing an *A. niger* phytase did not appear to decrease tibia ash (Table 1). However, to the best of our knowledge, phytases from different origins have only been compared *in vitro* in the context of Zn supplementation; no study comparing different phytase origins within one study is available *in vivo*.

When broilers and pigs were fed the same diets, the pH in the gizzard of broilers ranged from 4.0–4.3, whereas the pH in the

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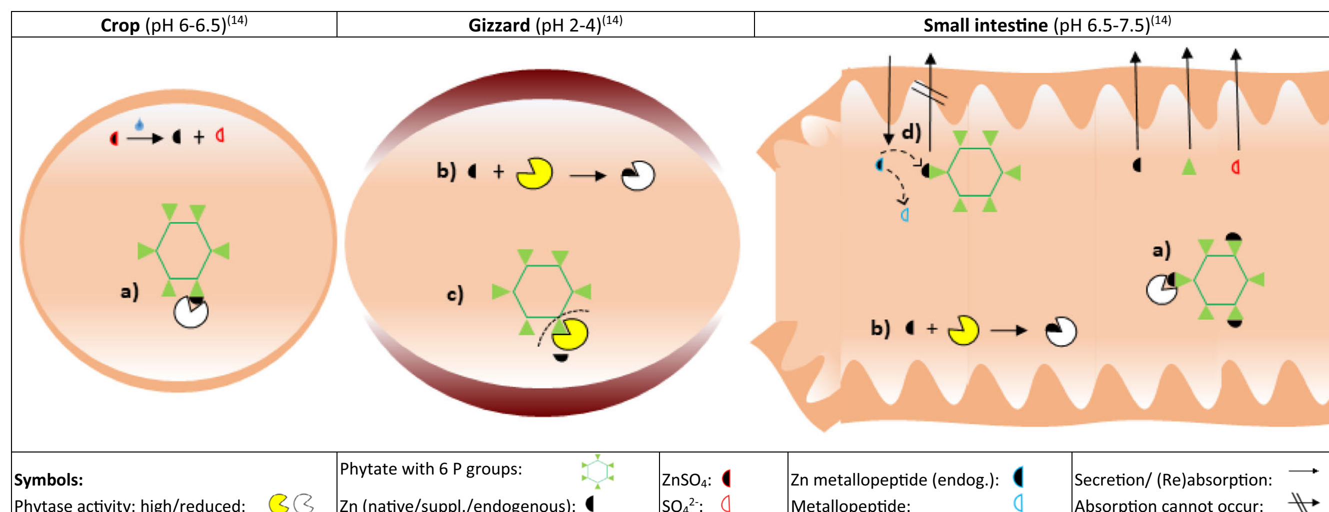

Figure 1. Suggested interactions of $InsP_6$ with Zn and phytase in crop, gizzard and small intestine of poultry. Crop: (a) indirect effect of Zn (native) via complex formation leading to reduced accessibility.[27] $ZnSO_4$ dissolves into Zn^{2+} and $SO_4{}^{2-}$ on contact with water. Gizzard: (b) direct effect of Zn (native/supplemental) on exogenous phytase.[29] (c) Exogenous phytase releases Zn (native/supplemental) from $InsP_6$.[53] Small intestine: (a) indirect effect of Zn (native/supplemental/endogenous) *via* complex formation leading to the reduced accessibility of $InsP_6$.[27] (b) Direct effect of Zn (native/supplemental/endogenous) on phytase (exogenous/endogenous).[29] (d) Endogenous Zn cannot be re-absorbed due to complex formation with $InsP_6$.[68]

stomach of pigs ranged from 5.0 to 5.2.[11] Although these pH values appear rather high for gastric pH, they exemplify the difference between the species when feeding the same diet. It was hypothesized that the lower gastric pH in broilers resulted in higher solubility of Zn-$InsP_6$ complexes, which would explain the lower effect of phytase supplementation on Zn bioavailability in broilers and the lower Zn requirement of broilers compared to pigs.[11] However, pH values vary in different parts of the stomach and vary over time depending on feed intake and composition,[42] and so average pH values should be interpreted with caution. A lower gastric pH in broilers compared to pigs would also explain the better performance of *E. coli* phytases, which have a lower optimum pH than *A. niger* phytases. By contrast, there appears to be no difference between *E. coli* and *A. niger* phytases in pigs, which have a higher gastric pH than broilers.[43] However, this assumption does not fit with the greater inhibition of *E. coli* compared to *A. niger* phytases by Zn in broilers. Further research on this topic is needed to gain an improved understanding of efficacy of phytase from different sources.

Pigs

Excessive Zn levels in broiler diets tend to have a negative effect on growth performance,[44] but pharmacological Zn levels (Zn doses of ≥ 1000 mg kg^{-1} Zn are defined herein as pharmacological) are commonly used to promote growth and improve gut health in weanling piglets.[4] As anticipated, several studies have reported lower P digestibility (or bone ash content) in pigs following pharmacological Zn dosing, especially in combination with phytase supplementation (Table 2).[27,45,46] The effects of Zn supplementation as Zn-methionine hydroxy analog chelate (ZnMet, 100 mg kg^{-1} Zn) or as ZnO (2000 mg kg^{-1} Zn) on total tract P digestibility changed.[47] The P digestibility was significantly higher when ZnMet was used irrespective of the phytase supplementation. It was suggested that ZnMet, as a chelated Zn source, was more stable than ZnO in the upper digestive tract of pigs. It was also hypothesized that the higher stability of ZnMet might have reduced the complexation of Zn, $InsP_6$, and Ca. However, the comparison among the Zn sources in that study was confounded by large differences in Zn dosage levels. In addition, batch effects of Zn can also occur. For chelated Zn compounds, the chelation strength that determines their stability, can differ between batches of the same product.[48] The chelation strength might affect *de novo* complex formation with $InsP_6$, because only the dissolved Zn can interact with $InsP_6$.[48] It has been proposed that chelated Zn compounds can be absorbed bound via amino acid transporters, giving chelated Zn compounds an alternative absorption pathway as opposed to inorganic Zn sources and reducing the potential for interaction of chelated Zn sources with $InsP_6$. However, this hypothesis has only been tested in cell culture and requires further validation.[49]

The assessment of Zn and $InsP_6$ cannot be done without considering Ca and its digestibility (defined as the proportion of Ca not excreted through feces). One hypothesis states that Zn competes with Ca for non-specific mineral transporters in the brush border membrane of the intestine. Because these transporters have a greater affinity for Zn than for Ca, Ca absorption might be reduced in the presence of excess Zn.[46] Therefore, it is more likely that Zn level, rather than Zn source, is the main factor responsible for the differences in Ca digestibility. Another study reported a decrease in Ca digestibility in the presence of ZnO supplementation (2400 mg kg^{-1} Zn) only in combined presence of phytase suggesting that excess concentrations of Zn increased the formation of insoluble Zn-Ca-$InsP_6$ complexes.[45] The observation of Zn effects on P and Ca digestibility being stronger in phytase-supplemented diets than in non-supplemented diets[27,45,46,50] might be the result of either a direct effect of Zn on phytase or an indirect effect via complex formation (Fig. 1), both of which are pH-dependent.

Overall, the effects of Zn supplementation on bone ash and P digestibility in pigs were inconsistent. The aforementioned studies differed in terms of phytase dosage, dietary $InsP_6$, P and Ca concentrations, and study duration (Table 2). However, which of these factors actually effect the P digestibility cannot be determined because of the limited data availability.

Different origins of phytase should be considered. Except for one study,[50] a negative effect of Zn supplementation on

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Table 2. Zn effects on phytase efficacy as determined by bone ash and P digestibility in pigs

Study	Experimental feeding (duration in days)	Native Zn (mg kg^{-1})	Targeted Zn supplement (mg kg^{-1})	Exogenous Zn source	Targeted phytase levels (FTU kg^{-1})	Phytase origin	Phytate-P (g kg^{-1})	Phosphorus (g kg^{-1})	Calcium (g kg^{-1})	Zn effect on: Bone ash (%)	Zn effect on: P digestibility (%)	Phytase × Zn
(27)	21	≈ 31	100 (+1500)	ZnO, $ZnCl_2$/ZnO	0, 500, 1000	*Escherichia coli*[c]	$2.5^{(cal.)}$	3.2	$7.0^{(cal.)}$	↓[f]	–	NS
(45)	20	≈ 31	120 (+1750, +3500)	$ZnSO_4$, ZnO	0, 2500	*Escherichia coli*[a]	2.2	6.8	8.9	–	↓[g,h]	[e,i]
(46)	13	≈ 16	125 (+2400)	$ZnSO_4$, ZnO	0, 1000, 3000	*Escherichia coli*[a]	≈ 1.3–1.4	4.3–3.6	7.7–6.3	–	↓[f,g]	[e]
(47)	42	P_1 ≈ 32, P_2 ≈ 31 P_3 ≈ 35	100, 2000	ZnMet, ZnO	0, 500	*Escherichia coli*[a]	$P_1 = 3.0^{(cal.)}$ $P_2 = 2.9^{(cal.)}$ $P_3 = 3.4^{(cal.)}$	P_1 = 5.8 P_2 = 5.5 P_3 = 5.0	P_1 = 6.6 P_2 = 6.7 P_3 = 5.0	NS	↓[g,j]	[e,l]
(50)	19	32	0, 20	$ZnSO_4$, ZnMet	0, 1200	*Aspergillus niger*[b]	$2.5^{(cal.)}$	7.6	9.5	NS	ZnMet: ↓[f,k] (*versus* $ZnSO_4$)	[e,l]
(64)	19	33	10, 25, 40[d]	$ZnSO_4$	O, 500	*Aspergillus niger*[b]	$2.9^{(cal.)}$	7.3	9.5	NS	–	NS
(63)	20	≈ 27	127 (+2000)	$ZnSO_4$, ZnO	0, 500	*Aspergillus niger*[b]	$P_1 = 2.4^{(cal.)}$ $P_2 = 2.9^{(cal.)}$	$P_1 = 7.9^{m,(cal.)}$ $P_2 = 6.9^{m,(cal.)}$	$P_1 = 9.0^{m,(cal.)}$ $P_2 = 8.0^{m,(cal.)}$	NS	–	NS
(68)	21	27–28	0, 100	$ZnSO_4$	0, 1500	*Aspergillus niger*[b]	$2.5^{(cal.)}$	5.0–5.3	8.3–7.9	–	NS[k]	NS

Note: Phytase × Zn, interaction of phytase and Zn; NS, not significant; ≈, subsequently calculated with table values based on diet formulation; cal., calculated; P_1, phase 1; P_2, phase 2; P_3, phase 3; underlining within a row associates Zn dosage with the relevant Zn source.

[a] Quantum Blue.
[b] Natuphos.
[c] Ecophos.
[d] Only relevant treatments are shown.
[e] Significant interaction.
[f] Only in presence of exogenous phytase.
[g] Apparent total tract digestibility.
[h] Effect greatest with phytase addition.
[i] Pharmacological Zn concentration, ZnO × P source was significant (phytase defined as P source).
[j] Effect of ZnO (2000 mg kg^{-1}) *versus* Zn methionine chelate [ZnMet (100 mg kg^{-1})], determined towards end P_2.
[k] Retained P.
[l] Significant interaction refers to P digestibility.
[m] Adjustment for phytase supplementation.

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Table 3. Phytase effects on Zn characteristics in poultry

Study	Experimental feeding (duration in days)	Native Zn (mg kg^{-1})	Targeted Zn supplement (mg kg^{-1})	Zn source	Targeted phytase levels (FTU kg^{-1})	Phytase origin	Phytate-P (g kg^{-1})	Phosphorus (g kg^{-1})	Calcium (g kg^{-1})	Phytase effect on bone Zn concentration	Phytase effect on Zn retention	Phytase × Zn
(11)	2–21	38	0, 15	$ZnSO_4$, ZnGly	0, 500	*Aspergillus niger*[a]	2.3	10.4	14.0	↑[f]	–	[e]
(26)	1–21	25	0, 2, 4, 8, 16	ZnPro	0, 500	i.m.	≈ 2.2	aP = 4.5	10.0[(cal.)]	↑	–	[e]
(28)	1–56	26	0, 30	ZnO	0, 500	*Escherichia coli*[b]	S ≈ 2.3, F ≈ 2.1	aP: S = 4.5, F = 4.3	S = 9.5, F = 9.2[(cal.)]	↑	NS	NS[g]
(30)	1–35	S = 26, F = 23	0, 15	$ZnSO_4$	0, 500	i.m.	S ≈ 2.3, F ≈ 2.1	aP: S = 5.0, F = 4.5	S = 9.9, F = 9.2	↑	–	NS
(53)	0–21	20	0[d]	–	0-600	*Aspergillus niger*[a]	2.1[(cal.)]	6.1	9.3	↑	↑	–
(54)	4–28	44	0, 10, 20, 40	$ZnSO_4$, ZnAA	0, 750	i.m.	3.5	8.1	9.2	↑[h]	–	[e]
(55)	0–16	35	0	–	0, 600	*Aspergillus ficuum*	≈ 2.2	≈ 7.2	≈ 7.3	↑	NS	–
(56)	0–42	S ≈ 34, G ≈ 31	60	i.m.	0–1000	*Schizosaccharomyces pombe*[c]	S = 3.1[(cal.)], G = 2.9[(cal.)]	S = 7.5–4.4[i] G = 6.2-4.4[i]	S = 9.5–7.0[i] G = 8.5–6.0[i]	↑[j]	–	–
(57)	1–21	53[(cal.)]	16	i.m.	0, 600	*Aspergillus niger*[a]	1.7[(cal.)]	5.0	13.0	NS	↑	–

Note: Phytase × Zn: interaction of phytase and Zn; NS, not significant; S, Starter; G, Grower; F, Finisher; aP, *calculated* 'available' phosphorus; i.m., information missing; ≈, subsequently calculated with table values; cal., calculated; ↑, sign. increase of bone Zn by phytase supplementation; ZnGly, Zn glycine chelate; ZnPro, Zn proteinate chelate; ZnAA, Zn amino acid chelate.
[a] Natuphos.
[b] 6-phytase, Danisco Animal Nutrition.
[c] Phyzyme® [produced by *Schizosaccharomyces pombe* (yeast)].
[d] Only relevant treatments are shown.
[e] Significant interaction.
[f] Only without Zn supplementation.
[g] Non-significant interaction refers to both, bone Zn and Zn retention.
[h] Increase in Zn concentration in ash only significant when Zn supplementation was ≤ 10 mg kg^{-1}.
[i] Adjustment for phytase supplementation.
[j] Only for highest phytase supplementation.

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Table 4. Phytase effects on Zn characteristics in pigs

Study	Experimental feeding (duration in days)	Native Zn ($mg\ kg^{-1}$)	Targeted Zn supplement ($mg\ kg^{-1}$)	Zn source	Targeted phytase levels ($FTU\ kg^{-1}$)	Phytase origin	Phytate-P ($g\ kg^{-1}$)	Phosphorus ($g\ kg^{-1}$)	Calcium ($g\ kg^{-1}$)	Phytase effect on bone Zn concentration	Phytase × Zn
(50)	19	32	0, 20	$ZnSO_4$, ZnMet	0, 1200	*Aspergillus niger*[a]	2.5[(cal.)]	7.6	9.5	↑[b]	NS
(64)	19	33	0, 10, 25, 40	$ZnSO_4$	0, 500	*Aspergillus niger*[a]	2.9[(cal.)]	7.3	9.5	↑	NS
(63)	20	≈ 27	127 (+2000)	$ZnSO_4$, ZnO	0, 500	*Aspergillus niger*[a]	P_1 = 2.4[(cal.)] P_2 = 2.9[(cal.)]	P_1 = 7.9[c (cal.)] P_2 = 6.9[c(cal.)]	P_1 = 9.0[c,(cal.)] P_2 = 8.0[c(cal.)]	NS	NS
(62)	19	30	0[d]	–	0–750	*Aspergillus niger*[a]	2.1[(cal.)]	6.8	9.6	↑	–

Note: Phytase × Zn: interaction of phytase and Zn; NS, not significant; ≈, subsequently calculated with table values; cal., calculated; P_1, phase 1; P_2, phase 2; underlining within a row associates Zn dosage with the relevant Zn source.
[a] Natuphos.
[b] Zn concentration in bone ash.
[c] Adjustment for phytase supplementation.
[d] Only relevant treatments are shown.

P-related traits was shown only in those studies involving an *E. coli* phytase. By contrast, no negative effects on bone ash or P digestibility were observed in studies with *A. niger* phytases.[14] However, more research on different phytase sources is needed to understand why *A. niger* phytases appear to be less affected by Zn supplementation compared to *E. coli* phytases. Other factors that differed among the trials did not follow a consistent pattern. Unexpectedly, the $InsP_6$-P concentration of the diet did not appear to influence the effects of pharmacological Zn supplementation. An effect of Zn on P digestibility was observed at both low (1.3 $g\ kg^{-1}$) and high (3.4 $g\ kg^{-1}$) concentration of $InsP_6$-P in the feed.[46,47]

In summary, the effects of Zn on bone ash were inconsistent in pigs and poultry, whereas the effects of Zn on P digestibility displayed a clearer picture. This suggests that direct measurements of P digestibility might be more appropriate than indirect measurements (bone ash data) for studying phytase-Zn-$InsP_6$ interactions. More research should be conducted on the disappearance of $InsP_6$ in the digestive tract, as this is a more suitable way to evaluate the effect of phytase on its substrate.

PHYTASE EFFECTS ON ZN TRAITS

Poultry

Bone Zn content

Among other factors, the Zn content of bones, which is the most important Zn deposit in the body, is commonly used as a trait to determine Zn bioavailability.[51] However, if the Zn supply exceeds the requirements of the bird, possible differences in Zn bioavailability cannot be detected because of the homeostasis. Supplementation with exogenous phytase increased the bone Zn content in cases of low Zn supply[52-56] except in one study (Table 3).[57] In the latter,[57] the maximum bone Zn content was probably achieved by Zn supplementation alone because the diet contained 70 $mg\ kg^{-1}$ Zn and the plateau of response was achieved at Zn levels between 48 and 85 $mg\ kg^{-1}$.[58] The increase in Zn bioavailability in the presence of exogenous phytase may reflect increased release of (native) Zn (Fig. 1)[57] or a diminished *de novo* formation of $InsP_6$-Zn-complexes following the increased $InsP_6$ degradation in the anterior digestive tract. The degradation products of $InsP_6$, less phosphorylated $InsP_x$, form weaker complexes with Zn than $InsP_6$, which might explain the decrease in the probability of stable *de novo* complexation in the presence of exogenous phytase.[59]

The dietary $InsP_6$ concentration might determine the dietary Zn level at which the bones are saturated with Zn, as Zn bioavailability is reduced with $InsP_6$ supplementation.[4,10] It was hypothesized that the re-absorption of endogenous Zn might be inhibited because of the enhanced complex formation of endogenous Zn and $InsP_6$ in the small intestine, rendering Zn unavailable for re-absorption (Fig. 1).[60] Accordingly, in rats, only 20% of endogenously secreted Zn was re-absorbed when the diet was supplemented with $InsP_6$, whereas 33% was re-absorbed without $InsP_6$ supplementation.[61]

Zn digestibility and retention

Approaches to determine Zn utilization include measuring Zn retention or prececal Zn digestibility; the proportion of Zn intake not recovered in the excreta or at the end of the small intestine. The results based on these responses are rare and inconsistent (Table 3). Although some studies have shown that phytase increased Zn retention,[57] others did not.[55,28] In 21-day-old broilers,

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prececal Zn digestibility was significantly increased in treatments with 500 or 750 FTU kg^{-1} phytase supplementation compared to treatments without phytase supplementation.[56] Whereas, in the same study, the addition of 1000 FTU kg^{-1} phytase resulted in significantly reduced prececal Zn digestibility. In 35-day-old broilers in that study, an increase in prececal Zn digestibility was observed at 750 or 1000 FTU kg^{-1} phytase supplementation, whereas the effect was not significant at 500 FTU kg^{-1} phytase supplementation.[56] It should be noted that the P and Ca levels of the feed in the study were adjusted with increasing phytase supplementation. Accordingly, it is not possible to distinguish between the effects of phytase and P and Ca supplementation.

Pigs

A few studies[62,63] were not included in the latest review on phytase effects on Zn bioavailability in pigs.[13] Consistent with that review, phytase supplementation increased Zn bioavailability using non-pharmacological Zn doses in most studies (Table 4). Phytase supplementation significantly increased bone Zn levels in pigs fed a diet that contained 33–73 mg kg^{-1} total Zn (and supplemented Zn as $ZnSO_4$),[64] whereas, at 70 mg kg^{-1} total Zn (supplemented Zn as ZnO), phytase supplementation did not significantly increase bone Zn.[63]

By contrast to $ZnSO_4$, ZnO is insoluble in water and requires acidic pH conditions (0.4% hydrochloric acid) for solubilization.[65] Therefore, in the stomach and the subsequent digestive tract, the susceptibility of ZnO and $ZnSO_4$ interaction with dietary antagonists such as $InsP_6$, which increases with increasing solubility, is probably similar.[66,67]

Differences in the levels of Ca and P supplementation might also have contributed to the differences in the aforementioned studies.[63,64] In the one study that did not measure an effect of phytase on Zn availability,[63] less Ca and P were supplemented than in the study in which a phytase effect on Zn availability was measured.[64] However, because phytase activity is known to be inhibited by high Ca concentrations, it is unlikely that this difference was relevant.

Native *versus* supplemented Zn

It remains unclear whether the native or supplemented Zn interacts with $InsP_6$ in the digestive tract. It has been hypothesized that the magnitude of the response to Zn supplementation in the presence of phytase is higher because of the interaction between supplemental Zn and $InsP_6$.[64] Because no interaction of supplemental Zn and phytase for bone Zn was observed, it was concluded that only native Zn interacted with $InsP_6$.[64] However, in that study, phytase supplementation of 700 FTU kg^{-1} to a non-Zn supplemented diet having 30 mg kg^{-1} native Zn concentration had a greater effect on bone Zn concentration than the supplementation with 30 mg Zn kg^{-1}. This suggested that exogenous phytase made more Zn available than just the dietary native Zn, and it was assumed that the re-absorption of endogenously secreted Zn increased with phytase supplementation.[64] However, the complex formation of supplemented Zn and $InsP_6$ might explain the differential effects of Zn and phytase supplementation on bone Zn content. The determination of prececal $InsP_6$ disappearance and Zn digestibility in future studies can help to gain better insight into these processes.

CONCLUSIONS AND RECOMMENDATIONS

Observations from the literature indicate that Zn and $InsP_6$ interact under certain conditions. Zn might influence $InsP_6$ degradation either via the complex formation of Zn and $InsP_6$ or via affecting phytase present in the digestive tract. Zn availability being increased by phytase supplementation suggests that the main mechanism is the complex formation of Zn and $InsP_6$ rather than Zn and phytase. This is because the latter would be expected to cause decreased Zn availability unless Zn binds to both $InsP_6$ and phytase. However, the limited number of *in vivo* studies does not allow us to assess whether an inhibitory effect of Zn on phytase efficacy occurs at all *in vivo*.

In poultry, the effects of Zn on phytase efficacy were inconsistent probably as a result of the lack of studies on responses other than tibia ash to evaluate phytase efficacy. In pigs, pharmacological Zn doses appear to decrease P digestibility. Phytase supplementation overall increased the bioavailability of native Zn in pigs and poultry with low supplies of dietary Zn. However, whether supplemented or native Zn interacts with native $InsP_6$ remains an unresolved issue.

Interactions of Zn with $InsP_6$ and phytase are influenced by multiple interacting factors, which cannot be fully assessed with the literature currently available. To gain deeper insight, identify the main mechanism, and enable meta-analysis in the future, new studies should provide comprehensive information about their experimental setup, including Zn source and composition, dietary native and supplemental Zn concentrations, concentrations and sources of dietary P and Ca, dietary $InsP_6$ concentration, phytase activity, and origin and concentrations of other minerals. To better understand the mechanisms related to Zn and phytase supplementation, it is recommended to include gastrointestinal $InsP_6$ degradation and P digestibility measurements in the list of response criteria.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

REFERENCES

1 Eeckhout W and De Paepe M, Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. *Anim Feed Sci Technol* **47**: 19–29 (1994).
2 Rodehutscord M, Sommerfeld V, Kühn I and Bedford MR, Phytases: potential and limits of phytate destruction in the digestive tract of pigs and poultry, in *Enzymes in Farm Animal Nutrition*, ed. by Bedford MR, Partridge GG, Hruby M and Walk CL. CABI GB, Wallingford, pp. 124–152 (2022).
3 Rigden DJ, The histidine phosphatase superfamily: structure and function. *Biochemistry* **409**:333–348 (2008).
4 Brugger D and Windisch WM, Strategies and challenges to increase the precision in feeding zinc to monogastric livestock. *Anim Nutr* **3**:103–108 (2017).
5 Maddiaiah VT, Kurnick AA and Reid BL, Phytic acid studies. *Proc Soc Exp Biol Med* **115**:391–393 (1964).
6 Maenz DD, Engele-Schaan CM, Newkirk RW and Classen HL, The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution

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and in a slurry of canola meal. *Anim Feed Sci Technol* **81**:177–192 (1999).
7 Erdman JW, Effects of soya protein on mineral availability. *J Am Oil Chem Soc* **58**:489–493 (1981).
8 Dionisio G, Uddin MN and Vincze E, Enrichment and identification of the most abundant zinc binding proteins in developing barley grains by zinc-IMAC capture and nano LC–MS/MS. *Proteomes* **6**:3 (2018).
9 Oberleas D, Muhrer ME and O'Dell BL, Effects of phytic acid on zinc availability and parakeratosis in swine. *J Anim Sci* **21**:57–61 (1962).
10 O'Dell BL and Savage JE, Effect of phytic acid on zinc availability. *Proc Soc Exp Biol Med* **103**:304–306 (1960).
11 Schlegel P, Nys Y and Jondreville C, Zinc availability and digestive zinc solubility in piglets and broilers fed diets varying in their phytate contents, phytase activity and supplemented zinc source. *Animal* **4**:200–209 (2010).
12 Singh PK, Significance of phytic acid and supplemental phytase in chicken nutrition: a review. *Worlds Poult Sci J* **64**:553–580 (2008).
13 Rimbach G, Pallauf J, Moehring J, Kraemer K and Minihane AM, Effect of dietary phytate and microbial phytase on mineral and trace element bioavailability – a literature review. *Curr Top Nutraceutical Res* **6**:131–144 (2008).
14 Santos T, Connolly C and Murphy R, Trace element inhibition of phytase activity. *Biol Trace Elem Res* **163**:255–265 (2015).
15 Kristoffersen S, Itani K, Benzertiha A, Kierończyk B, Kjos NP and Svihus B, Effect of crop retention time and acidification of the feed on phytase efficacy in broiler chickens. *Br Poultry Sci* **62**:443–451 (2021).
16 Menezes-Blackburn D, Gabler S and Greiner R, Performance of seven commercial phytases in an in vitro simulation of poultry digestive tract. *J Agric Food Chem* **63**:6142–6149 (2015).
17 Brejnholt SM, Dionisio G, Glitsoe V, Skov LK and Brinch-Pedersen H, The degradation of phytate by microbial and wheat phytases is dependent on the phytate matrix and the phytase origin. *J Sci Food Agric* **91**:1398–1405 (2011).
18 O'Dell BL and de Boland A, Complexation of phytate with proteins and cations in corn germ and oil seed meals. *J Agric Food Chem* **24**:804–808 (1976).
19 Scheuermann SE, Lantzsch H-J and Menke KH, In vitro und in vivo Untersuchungen zur Hydrolyse von Phytat: I. Löslichkeit von Phytat. *J Anim Physiol Anim Nutr* **60**:55–63 (1988).
20 Leske KL and Coon CN, A bioassay to determine the effect of phytase on phytate phosphorus hydrolysis and total phosphorus retention of feed ingredients as determined with broilers and laying hens. *Poult Sci* **78**:1151–1157 (1999).
21 Krieg J, Siegert W, Berghaus D, Bock J, Feuerstein D and Rodehutscord M, Phytase supplementation effects on amino acid digestibility depend on the protein source in the diet but are not related to InsP6 degradation in broiler chickens. *Poult Sci* **99**:3251–3265 (2020).
22 Reddy NR, Sathe SK and Salunkhe DK, Phytates in legumes and cereals. *Adv Food Res* **28**:1–92 (1982).
23 Haese E, Titze N and Rodehutscord M, In situ ruminal disappearance of crude protein and phytate from differently processed rapeseed meals in dairy cows. *J Sci Food Agric* **102**:2805–2812 (2022).
24 Selle PH, Walker AR and Bryden WL, Total and phytate-phosphorus contents and phytase activity of Australian-sourced feed ingredients for pigs and poultry. *Aust J Exp Agric* **43**:475–479 (2003).
25 Künzel S, Borda-Molina D, Zuber T, Hartung J, Siegert W, Feuerstein D *et al*., Relative phytase efficacy values as affected by response traits, including ileal microbiota composition. *Poult Sci* **100**:101133 (2021).
26 Ao T, Pierce JL, Pescatore AJ, Cantor AH, Dawson KA, Ford MJ *et al*., Effects of organic zinc and phytase supplementation in a maize-soybean meal diet on the performance and tissue zinc content of broiler chicks. *Br Poultry Sci* **48**:690–695 (2007).
27 Augspurger NR, Spencer JD, Webel DM and Baker DH, Pharmacological zinc levels reduce the phosphorus-releasing efficacy of phytase in young pigs and chickens. *J Anim Sci* **82**:1732–1739 (2004).
28 Attia YA and Addeo NF, Al-Hamid AA-HEA and Bovera F, effects of phytase supplementation to diets with or without zinc addition on growth performance and zinc utilization of white Pekin ducks. *Animals* **9**:280 (2019).
29 Maret W, Inhibitory zinc sites in enzymes. *Biometals* **26**:197–204 (2013).
30 Roy A, Mandal GP, Pal K, Samanta I, Biswas P and Roy B, Effect of zinc supplementation with or without phytase on performance, mineral accumulation in tissues and immune response of broiler. *Anim Nutr and Feed Tech* **14**:311–319 (2014).
31 Mohanna C and Nys Y, Changes in zinc and manganese availability in broiler chicks induced by vegetal and microbial phytases. *Anim Feed Sci Technol* **77**:241–253 (1999).
32 Shastak Y, Witzig M, Hartung K, Bessei W and Rodehutscord M, Comparison and evaluation of bone measurements for the assessment of mineral phosphorus sources in broilers. *Poult Sci* **91**:2210–2220 (2012).
33 Ammerman CB, Douglas CR, Davis GK and Harms RH, Comparison of phosphorus availability assay techniques for chicks. *Poult Sci* **40**: 548–553 (1961).
34 Rodehutscord M, Dieckmann A, Witzig M and Shastak Y, A note on sampling digesta from the ileum of broilers in phosphorus digestibility studies. *Poult Sci* **91**:965–971 (2012).
35 Dersjant-Li Y, Awati A, Schulze H and Partridge G, Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *J Sci Food Agric* **95**:878–896 (2014).
36 Anwar MN, Ravindran V, Morel PC, Ravindran G and Cowieson AJ, Apparent ileal digestibility of calcium in limestone for broiler chickens. *Anim Feed Sci Technol* **213**:142–147 (2016).
37 Akter M, Iji PA and Graham H, Increased iron level in phytase-supplemented diets reduces performance and nutrient utilisation in broiler chickens. *Br Poultry Sci* **58**:409–417 (2017).
38 Krieg J, Borda-Molina D, Siegert W, Sommerfeld V, Chi YP, Taheri HR *et al*., Effects of calcium level and source, formic acid, and phytase on phytate degradation and the microbiota in the digestive tract of broiler chickens. *Animal microbiome* **3**:1–18 (2021).
39 Fordyce EJ, Forbes RM, Robbins KR and Erdman JW, Phytate × calcium/zinc molar ratios: are they predictive of zinc bioavailability? *J Food Sci* **52**:440–444 (1987).
40 Bafundo KW, Baker DH and Fitzgerald PR, Zinc utilization in the chick as influenced by dietary concentrations of calcium and phytate and by Eimeria acervulina infection. *Poult Sci* **63**:2430–2437 (1984).
41 Lönnerdal B, Dietary factors influencing zinc absorption. *J Nutr* **130**: 1378s–1383s (2000).
42 Lee SA, Dunne J, Febery E, Wilcock P, Mottram T and Bedford MR, Superdosing phytase reduces real-time gastric pH in broilers and weaned piglets. *Br Poultry Sci* **59**:330–339 (2018).
43 Augspurger NR, Webel DM, Lei XG and Baker DH, Efficacy of an *E. coli* phytase expressed in yeast for releasing phytate-bound phosphorus in young chicks and pigs. *J Anim Sci* **81**:474–483 (2003).
44 Roberson RH and Schaible PJ, The tolerance of growing chicks for high levels of different forms of zinc. *Poult Sci* **39**:893–896 (1960).
45 Walk CL, Wilcock P and Magowan E, Evaluation of the effects of pharmacological zinc oxide and phosphorus source on weaned piglet growth performance, plasma minerals and mineral digestibility. *Animal* **9**:1145–1152 (2015).
46 Blavi L, Sola-Oriol D, Perez JF and Stein HH, Effects of zinc oxide and microbial phytase on digestibility of calcium and phosphorus in maize-based diets fed to growing pigs. *J Anim Sci* **95**:847–854 (2017).
47 Ren P, Chen J, Wedekind K, Hancock D and Vázquez-Añón M, Interactive effects of zinc and copper sources and phytase on growth performance, mineral digestibility, bone mineral concentrations, oxidative status, and gut morphology in nursery pigs. *Transl Anim Sci* **4**:783–798 (2020).
48 Byrne L, Hynes MJ, Connolly CD and Murphy RA, Influence of the chelation process on the stability of organic trace mineral supplements used in animal nutrition. *Animals* **11**:1730 (2021).
49 Sauer AK, Pfaender S, Hagmeyer S, Tarana L, Mattes A-K, Küry S *et al*., Characterization of zinc amino acid complexes for zinc delivery in vitro using Caco-2 cells and entereocytes from hiPSC. *Biometals* **30**:643–661 (2017).
50 Revy P, Jondreville C, Dourmad J and Nys Y, Effect of zinc supplemented as either an organic or an inorganic source and of microbial phytase on zinc and other minerals utilisation by weanling pigs. *Anim Feed Sci Technol* **116**:93–112 (2004).
51 Wedekind KJ, Hortin AE and Baker DH, Methodology for assessing zinc bioavailability: efficacy estimates for zinc-methionine, zinc sulfate, and zinc oxide. *J Anim Sci* **70**:178–1787 (1992).
52 Schlegel P, Sauvant D and Jondreville C, Bioavailability of zinc sources and their interaction with phytates in broilers and piglets. *Animal* **7**: 47–59 (2013).

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53 Yi Z, Kornegay ET and Denbow DM, Supplemental microbial phytase improves zinc utilization in broilers. *Poult Sci* **75**:540–546 (1996).
54 Świątkiewicz S, Koreleski J and Zhong D, The bioavailability of zinc from inorganic and organic sources in broiler chickens as affected by addition of phytase. *J Anim Feed Sci* **10**:317–328 (2001).
55 Roberson KD and Edwards HM, Effects of 1,25-dihydroxycholecalciferol and phytase on zinc utilization in broiler chicks. *Poult Sci* **73**:1312–1326 (1994).
56 Santos FR, Hruby M, Pierson E, Remus JC and Sakomura NK, Effect of Phytase supplementation in diets on nutrient digestibility and performance in broiler chicks. *J Appl Poultry Res* **17**:191–201 (2008).
57 Sebastian S, Touchburn SP, Chavez ER and Lague PC, The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper, and zinc in broiler chickens fed corn-soybean diets. *Poult Sci* **75**:729–736 (1996).
58 Mohanna C and Nys Y, Effect of dietary zinc content and sources on the growth, body zinc deposition and retention, zinc excretion and immune response in chickens. *Br Poultry Sci* **40**:108–114 (1999).
59 Persson H, Türk M, Nyman M and Sandberg A-S, Binding of Cu2+, Zn2+, and Cd2+ to inositol tri-, tetra-, penta-, and hexaphosphates. *J Agric Food Chem* **46**:3194–3200 (1998).
60 Babatunde OO, Jendza JA, Ader P, Xue P, Adedokun SA and Adeola O, Response of broiler chickens in the starter and finisher phases to 3 sources of microbial phytase. *Poult Sci* **99**:3997–4008 (2020).
61 Davies NT and Nightingale R, The effects of phytate on intestinal absorption and secretion of zinc, and whole-body retention of Zn, copper, iron and manganese in rats. *Br J Nutr* **34**:243–258 (1975).
62 Jondrevillea C, Hayler R and Feuerstein D, Replacement of zinc sulphate by microbial phytase for piglets given a maize-soya-bean meal diet. *Anim Sci* **81**:77–83 (2005).
63 Williams SB, Southern LL and Bidner TD, Effects of supplemental dietary phytase and pharmacological concentrations of zinc on growth performance and tissue zinc concentrations of weanling pigs. *J Anim Sci* **83**:386–392 (2005).
64 Revy PS, Jondreville C, Dourmad JY and Nys Y, Assessment of dietary zinc requirement of weaned piglets fed diets with or without microbial phytase. *J Anim Physiol Anim Nutr* **90**:50–59 (2006).
65 Sandoval M, Henry PR, Ammerman CB, Miles RD and Littell RC, Relative bioavailability of supplemental inorganic zinc sources for chicks. *J Anim Sci* **75**:3195–3205 (1997).
66 Batal AB, Parr TM and Baker DH, Zinc bioavailability in tetrabasic zinc chloride and the dietary zinc requirement of young chicks fed a soy concentrate diet. *Poult Sci* **80**:87–90 (2001).
67 Susaki H, Matsui T, Ashida K-Y, Fujita S, Nakajima T and Yano H, Availability of a zinc amino acid chelate for growing pigs. *Nihon Chikusan Gakkaiho* **70**:124–128 (1999).
68 Adeola O, Lawrence BV, Sutton AL and Cline TR, Phytase-induced changes in mineral utilization in zinc-supplemented diets for pigs. *J Anim Sci* **73**:3384–3391 (1995).

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