EFFECTS OF COBALT AND NICKEL ON ZINC AVAILABILITY IN CHICKS AND PIGS FED PRACTICAL-TYPE DIETS HIGH IN CALCIUM\textsuperscript{1}

A. S. Chung\textsuperscript{2,3}, S. T. Lee\textsuperscript{3}, R. H. Grummer\textsuperscript{a} and W. G. Hoekstra\textsuperscript{b,c}

Departments of Biochemistry\textsuperscript{b}, Nutritional Sciences\textsuperscript{c}, and Meat and Animal Science\textsuperscript{a}
University of Wisconsin-Madison
Madison, Wisconsin 53706, USA

Summary

Experiments were conducted with chicks and pigs to determine whether the sparing effects of cobalt (Co) or nickel (Ni) on zinc (Zn) nutrition were due to an improved Zn availability. They included a Zn balance study with New Hampshire X Leghorn cross chicks, a \textsuperscript{65}Zn absorption study with Ancona chicks and a \textsuperscript{65}Zn balance study with pigs. The basal diet was a corn-soybean type diet high in calcium. In the Zn balance study with New Hampshire X Leghorn cross chicks, Zn retention percentages for basal, + 54 ppm Ni or + 54 ppm Co were 39.4, 40.4 and 48.3, respectively. In the \textsuperscript{65}Zn absorption study with chicks, adding these levels of Ni or Co to the basal diet increased \textsuperscript{65}Zn absorption from 12.8% to 14.8% and 15.1%, respectively. Supplementation of Ni and Co increased the proportion of body \textsuperscript{65}Zn found in liver and bone. With the pig experiment, \textsuperscript{65}Zn retention percentages for basal, + 54 ppm Co and + 60 ppm Zn groups were 20.0, 26.7 and 12.2, respectively; while Zn retention values (mg) in the body were 29.5, 45.1 and 60.5, respectively. In addition, supplemental Co increased \textsuperscript{65}Zn concentration in the blood, liver, kidney and duodenum. These studies showed that supplementation of the basal diet with Co resulted in increased absorption of dietary Zn in chicks and pigs. The effect of Ni in chicks was less than that of Co. The ability of supplemental Co and to a lesser extent of Ni to improve weight gain as well as reduce other Zn-deficient signs in both species led to the practical corn-soybean type diet high in calcium can be explained, at least in part by an associated increase in Zn absorption. The possible mechanism involved in these effects are discussed.

(Key Words: Ni or Co on Zn Availability)

Introduction

Studies in vitro of zinc metalloenzymes have shown that cobalt or nickel can substitute for zinc in numerous purified zinc metalloenzymes and in vivo cobalt can replace zinc in the D-lactic dehydrogenase of yeast (Curdel, 1966), alcohol dehydrogenase of yeast (Curdel and Iwatsube, 1968), and the RNA polymerase of E. coli (Speckhard et al., 1977). In animal experiments\textsuperscript{4-6}, supplements of cobalt or nickel have improved weight gain and alleviated other zinc deficiency signs in pigs and chickens fed corn-soybean type diets containing a high calcium level of 1.3 and 1.5% respectively. Such sparing effects of cobalt or nickel on zinc nutrition could not be demonstrated in chicks fed a normal calcium, semipurified diet (Hoekstra, unpublished data) nor were the effects shown for cobalt or nickel in the case of rats fed a semipurified diet of 0.7 or 1.2% calcium content (Chung, unpublished data). These results indicated that differences in the ability of cobalt or nickel to spare zinc in animals were probably due to the variation in the diet rather than species. Because supplemental cobalt or nickel increased zinc content in tissues of pigs, especially in the serum\textsuperscript{5}, it seems likely that in animals fed certain types of

\textsuperscript{1}Presented in part at the annual meeting of the Federation of American Societies of Experimental Biology and published in abstract form in Federation Proceedings (Abstr.) 37:668, 1978.

\textsuperscript{2}Address reprint requests to Dr. A.S. Chung, Department of Biology, Korea Institute of Technology, Taejon 300-31, Korea.

\textsuperscript{3}Department of Biology, Korea Institute of Technology, Taejon 300-31, Korea.

\textsuperscript{4}Received January 21, 1988

\textsuperscript{5}Accepted July 26, 1988

81 AJAS 1988 Vol. 1 (No. 2) 81 – 88
diets cobalt or nickel exert their effect by increasing zinc availability rather than substituting for zinc in zinc-dependent enzymes.

In view of the above, three experiments were conducted with chicks and pigs to determine whether supplemental cobalt or nickel can cause increased zinc absorption from practical-type diets containing 1.3-1.5% calcium. The experiments involved an assessment of zinc balance in chicks and the absorption of tritium doses of $^{65}$Zn in chicks and pigs.

Materials and Methods

Two experiments were conducted with day-old chicks and a third with weaning pigs. Chicks were assigned randomly to treatments without segregation by sex and caged in a stainless steel battery with temperatures ranging from 40°C at the start to 32°C at the end of the experiment. Pigs were allotted to treatments according to weaning weight and litter. New Hampshire X Single Comb White Leghorn cross chicks were used in the first experiment, and Ancona chicks, special inbred line and high requirement of Zn for feathering, were used in the second experiment. Chester White and Yorkshire pigs were employed in the third experiment.

In the experiments with chicks, feed and distilled water were supplied ad libitum in aluminum and stainless steel troughs, respectively. The same basal chick diet (corn-soybean type diet containing 1.5% calcium) as described previously was used. Pigs were housed in adjoining wooden pens with concrete floors and received their respective diets ad libitum from wooden feeders. Tap water was available ad libitum from iron troughs. The pigs were put in metabolism crates prior to the administration of $^{65}$Zn. The basal diet for pigs was described previously.

The chick experiments included seven treatment groups: (1) basal; (2) basal + 54 ppm nickel; (3) basal + 54 ppm cobalt; (4) basal + 54 ppm nickel + 54 ppm cobalt; (5) basal + 60 ppm cobalt; (6) basal + 60 ppm zinc + 54 ppm cobalt; (7) basal + 60 ppm zinc + 54 ppm cobalt (the amounts of Co, Ni and Zn are equimolar). Ten New Hampshire X Leghorn cross chicks and 15 Ancona chicks were used in each treatment group except for treatment 7 in experiment 1, in which 9 New Hampshire X Leghorn cross chicks were used, and treatment 1 in experiment 2, in which 13 chicks were used. In experiment 1, chicks were fed their respective diets for 18 days and then the combined feces and urine of each treatment group were collected for three days on a sheet of aluminum foil which was put under the wire screen of the battery. Zinc retention was assessed by analysis for zinc in the diet and excreta. The growth rate and feather scores of the first experiment were reported in another paper.

Zn absorption studies were performed in experiment 2 by the method of Heth and Hoekstra (1965). Chicks were fed their respective diets ad libitum for 13 days. After a 12-hour fast, 10 chicks from each group (except 9 chicks for treatment 1) on a per chick basis, were offered 3 g of their appropriate diet to which had been added 2 μCi $^{65}$ZnCl$_2$. The remaining chicks (4 chicks for treatment 1) in each treatment group were injected intramuscularly into the right thigh with about 1 μCi $^{65}$Zn per chick as a zinc-glycine (1:4) at pH 7.4. Body $^{65}$Zn was determined in a whole animal gamma scintillation counter every 4 or 5 hours initially and every 24 hours later. The percentage of $^{65}$Zn absorption was calculated as outlined by Heth and Hoekstra (1965). Twelve days after the $^{65}$Zn dosing, the chicks were weighed and killed by decapitation. Livers, breast muscle and tibia were placed into individual counting vials, weighed and assayed for $^{65}$Zn in a crystal scintillation counter.

The Zn concentrations for individual tissues were corrected for differences in body weight and final whole body $^{65}$Zn activity using the following formula: corrected cpm = (tissue cpm/g

7Poultry Research Laboratory, University of Wisconsin, Madison, USA.
8Experimental Station, Arlington Farm, Arlington, Wisconsin, USA.
9New England Nuclear, Boston, Massachusetts.
tissue/total body cpm) x g body weight x 100. Body weight and feather scores were obtained at the end of each experiment. Feather scores were based on visual inspection and ranged from 0 for normal to 5 for severely frayed and broken feathers as described by Nielsen (1968).

Experiment 3 conducted with pigs involved total collection of feces in metabolism crates to assess the effect of dietary cobalt on the absorption of orally administered $^{65}$Zn. Because the number of metabolism crates was limited, pigs for these studies were representative of larger groups of pigs started on diets at four different times (referred to as replicates). Each of the first three replicates compared one castrated male Chester White pig from each of three treatment groups: (1) basal, (2) basal + 54 ppm cobalt and (3) basal + 60 ppm zinc, while the fourth replicate compared 2 male Yorkshire pigs from each of treatment groups 1 and 2 but did not include the zinc-supplemented group. Therefore, in assessing the effects of cobalt, there were measurements made on a total of 5 pigs per treatment group. In replicates 1-4, the pigs had been fed the diets for 6, 5, 11 and 3 weeks, respectively, before they were placed in metabolism crates.

The metabolism crates were housed in a well insulated and temperature controlled room and were basically of the Shinfield design (Farre et al., 1968). The crates were made of stainless steel bars and galvanized metal clamps which were coated by several layers of perma clear acrylic to prevent access to zinc. The floors were made of stainless steel screen covered with plastic screen, the feed troughs of stainless steel and the urine funnels of fiber glass. Mosquito net was used at the top of the urine funnels to prevent contamination of the urine with feces.

Pigs were put in metabolism crates for 10 to 14 days as an adjustment period. During the study period, the pigs in each replicate were of similar body weights and were fed the same amount of feed as determined by the feed intake of the basal group.

$^{65}$ZnCl$_2$ (200 μCi) mixed with 250 or 500 g of the appropriate diet was fed to each pig after fasting overnight.Samples of feed were assayed for $^{65}$Zn in a crystal scintillation counter to determine the $^{65}$Zn intake. Blood samples were taken from the jugular vein at 3, 6, 10 and 24 hours (except for the lack of a 10-hour sample of replication 1) and at subsequent 24-hour intervals for an additional 6 days. Moistened feed was fed for an hour twice a day because the basal pigs at the moistened feed more and faster than a dry feed. Additional distilled water was supplied to the pigs at the end of feeding. Urine and feces were collected once a day for the 7-day metabolism period. Each day's collection of feces in plastic bags and the urine in plastic jars were stored at 4°C. Ten ml urine samples were placed in scintillation vials for determination of $^{65}$Zn. Feces were ground three times with a meat grinder and after mixing, weighed samples were placed in scintillation vials using a 10 ml plastic syringe with the tip end cut off. At the end of the 7-day collection period, the pigs were weighed, killed and the liver, kidney, duodenum and muscle from the ham were sampled for later determination of $^{65}$Zn. The percentage of the $^{65}$Zn intake which was present in blood at any given time was calculated by assuming that blood comprised 8 percent of the body weight.

$^{65}$Zn concentration in tissues

$$\frac{^{65}Zn\text{ concentration in tissues}}{^{65}Zn/\text{g tissue} \times \text{body weight}} = \frac{^{65}Zn\text{ intake}}{X\text{ 100}}$$

The percentage retention of Zn was calculated for the 7-day metabolism trial as follows:

$$\%^{65}\text{Zn retained} = \frac{\text{total intake}^{65}\text{Zn} - \text{fecal}^{65}\text{Zn} - \text{urinary}^{65}\text{Zn}}{\text{total intake of}^{65}\text{Zn}} \times 100$$

Total Zn retention in mg was estimated by multiplying the total intake of Zn by the percentage retention of $^{65}$Zn, because stable Zn was not determined in the urine and feces.

Nickel, cobalt and zinc were supplemented as carbonate salts. Diet samples were ashed overnight at a temperature not exceeding 580°C and were analyzed for Zn by an atomic absorption
spectrophotometer\textsuperscript{15} according to standard procedure. The data were analyzed statistically using Duncan's multiple range test as modified by Kramer and using the student's t test (Steel and Torrie, 1960). Feather scores were not tested statistically since the visual inspection could yield only an integer value.

**Results**

Results of the stable zinc balance study with chicks are in table 1. A supplementation of 54 ppm cobalt to a basal diet increased the absolute quantity of zinc retained as well as the percentage of dietary zinc retained. Statistical analysis was not possible because the experiment was done with groups of chicks, not individual chicks. Nickel added to the basal diet had little if any effect on zinc retention while the combination of cobalt and nickel produced results similar to cobalt alone.

Neither cobalt nor nickel affected zinc retention substantially when the diet was supplemented with 60 ppm zinc. As expected, the zinc sufficiently supplemented groups showed lower percentages of zinc retention than the zinc deficient groups regardless of supplementation with cobalt or nickel. However, total zinc retention of the zinc sufficient group was more than twice that of the zinc deficient groups. Cobalt added to the basal diet significantly (\( P < .05 \)) increased weight gain and reduced feather defects (data presented in a separate paper\textsuperscript{4}), while nickel was ineffective according to these criteria.

In experiment 2, the cobalt supplemented basal diet fed to Ancona chicks, was associated with an increased weight gain (table 2), reduced incidence of feather defects and increased \(^{65}\text{Zn} \) absorption. Nickel produced a similar but some-

<table>
<thead>
<tr>
<th>TABLE 2. WEIGHT GAIN, FEATHER SCORE AND (^{65}\text{Zn} ) ABSORPTION OF ANCONA CHICKS (EXPERIMENT 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Basal (B)</td>
</tr>
<tr>
<td>B + 54 ppm Ni</td>
</tr>
<tr>
<td>B + 54 ppm Co</td>
</tr>
<tr>
<td>B + 54 ppm Ni + 54 ppm Co</td>
</tr>
<tr>
<td>B + 60 ppm Zn</td>
</tr>
<tr>
<td>B + 60 ppm Zn + 54 ppm Ni</td>
</tr>
<tr>
<td>B + 60 ppm Zn + 54 ppm Co</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Mean ± S.E., column means with different superscripts are significantly different (\( P < .05 \)).

what smaller response in these observations. Neither cobalt nor nickel affected the parameters measured when added to the diet supplemented with 60 ppm zinc. The zinc sufficient groups showed lower (\( P < .05 \)) \(^{65}\text{Zn} \) absorptions than the zinc deficient groups regardless of the supplementation of cobalt or nickel. As shown in table 3 for the basal chicks, supplemental nickel and cobalt or the combination of nickel and cobalt tended to increase the proportion of the body \(^{65}\text{Zn} \) found in liver and bone but not that found in muscle. A similar effect from those of cobalt or nickel when added to the zinc supplemented

\textsuperscript{15} Atomic Absorption Spectrophotometer Model 405, Perkin Elmer Corp., Norwalk, Connecticut.

84
EFFECT OF CO AND NI ON ZN AVAILABILITY IN CHICKS AND PIGS

TABLE 3. $^{65}$Zn CONCENTRATION OF LIVER, MUSCLE AND TIBIA OF ANCONA CHICKS (EXPERIMENT 2)

<table>
<thead>
<tr>
<th></th>
<th>Corrected cpm/g tissue (see text)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Muscle</td>
<td>Bone</td>
<td>Liver</td>
</tr>
<tr>
<td>Basal (B)</td>
<td>90 ± 3$^b$$^1$</td>
<td>49 ± 2$^a$</td>
<td>126 ± 7$^d$</td>
<td></td>
</tr>
<tr>
<td>B + 54 ppm Ni</td>
<td>100 ± 2$^a$</td>
<td>47 ± 2$^a$</td>
<td>160 ± 12$^d$</td>
<td></td>
</tr>
<tr>
<td>B + 54 ppm Co</td>
<td>101 ± 3$^a$</td>
<td>43 ± 2$^a$</td>
<td>156 ± 9$^d$</td>
<td></td>
</tr>
<tr>
<td>B + 54 ppm Ni + 54 ppm Co</td>
<td>105 ± 3$^a$</td>
<td>46 ± 2$^a$</td>
<td>182 ± 9$^c$</td>
<td>182 ± 9$^c$</td>
</tr>
<tr>
<td>B + 60 ppm Zn</td>
<td>69 ± 5$^c$</td>
<td>24 ± 1$^b$</td>
<td>314 ± 10$^b$</td>
<td></td>
</tr>
<tr>
<td>B + 60 ppm Zn + 54 ppm Ni</td>
<td>69 ± 2$^c$</td>
<td>22 ± 1$^b$</td>
<td>350 ± 12$^a$</td>
<td>350 ± 12$^a$</td>
</tr>
<tr>
<td>B + 60 ppm Zn + 54 ppm Co</td>
<td>66 ± 2$^c$</td>
<td>25 ± 3$^b$</td>
<td>353 ± 13$^a$</td>
<td>353 ± 13$^a$</td>
</tr>
</tbody>
</table>

$^1$Mean ± S.E., column means within tissues with different superscripts are significantly different (P < .05).

In the pig metabolism studies (experiment 3, table 4), supplementation of the basal diet with cobalt increased significantly (P < .05, by the paired student's t test), both the total stable zinc retention and percentage $^{65}$Zn retention. However, the increase in total stable zinc retention was not significant by Duncan's new multiple range test.

Again as expected, supplementation with 60 ppm zinc decreased significantly (P < .05) $^{65}$Zn percentage retention but increased the absolute amount of zinc retained compared to basal and cobalt supplemented groups. This observation was similar to the result of the chick experiments.

Blood $^{65}$Zn of the pigs (figure 1) was increased dramatically as a result of supplementation of the basal diet with cobalt. The increased blood $^{65}$Zn in pigs fed the cobalt supplemented diet was evident at 3 hours and the difference between basal and cobalt supplemented groups was maximal at about 49 hours after $^{65}$Zn oral administration. Thereafter, the curves representing the $^{65}$Zn levels in blood of the two groups were essentially parallel. $^{65}$Zn levels in blood of pigs supplemented with 60 ppm zinc were similar to those of the basal group during the first 24 hours, but after that time the Zn levels of the zinc supplemented groups decreased compared to the basal group.

Supplementation of the basal diet of pigs with cobalt yielded significantly (P < .05) increased $^{65}$Zn concentration in the liver, kidney, intestine and muscle. Zinc supplementation with 60 ppm zinc decreased the $^{65}$Zn concentration in these tissues as expected, because of the large isotope

TABLE 4. TOTAL STABLE ZINC RETENTION AND PERCENTAGE $^{65}$Zn RETENTION FOR A 7-DAY METABOLISM STUDY WITH PIGS (EXPERIMENT 1)

<table>
<thead>
<tr>
<th>Replication</th>
<th>Stable zinc retention (mg)</th>
<th>$^{65}$Zn retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>B + 54 ppm Co</td>
</tr>
<tr>
<td>1</td>
<td>33.2</td>
<td>41.3</td>
</tr>
<tr>
<td>2</td>
<td>24.1</td>
<td>30.5</td>
</tr>
<tr>
<td>3</td>
<td>29.3</td>
<td>47.0</td>
</tr>
<tr>
<td>4-1</td>
<td>17.8</td>
<td>53.8</td>
</tr>
<tr>
<td>4-2</td>
<td>43.4</td>
<td>53.0</td>
</tr>
<tr>
<td>Mean S.E.</td>
<td>29.5 ± 4.3$^b$$^1$</td>
<td>45.1 ± 4.3$^ab$</td>
</tr>
</tbody>
</table>

$^1$Row means within same criteria, with different superscripts are significantly different (P < .05) according to Duncan's new multiple range test.

*Paired Student's test showed that supplemental Co improved significantly (P < .05) both stable Zn retention and $^{65}$Zn retention (%) compared to the basal group.
Discussion

Each of the experiments conducted with pigs and chicks fed the practical-type diet high in calcium showed that supplemental cobalt increased zinc absorption or retention. The increased total zinc retention found in the balance study with chicks (experiment 1) might be explained in part by the increased feed intake. However, cobalt increased total zinc retention by about 1.73 times and feed intake by about 1.38 times over basal, suggesting that cobalt increased zinc availability in addition to increasing feed intake. Further studies with Ancona chicks showed that cobalt supplementation to the basal diet did in fact increase $^{65}$Zn absorption. This effect has been confirmed with New Hampshire X Leghorn cross chicks. The effects of nickel on zinc absorption and retention have been less consistent and less dramatic than those with cobalt, but usually in the same direction. It seems that nickel is less effective than cobalt in improving zinc utilization. Improved zinc availability is, at least in part, responsible for improved weight gain and reduced feather defects caused by cobalt, and to a lesser extent nickel in chicks fed such diets which were suboptimal in zinc content high in calcium and comprised of conventional ingredients.

Pigs fed the cobalt supplemented diet showed increased $^{65}$Zn retention, a greater proportion of the dietary $^{65}$Zn in the blood, and a higher $^{65}$Zn concentration in liver, kidney, muscle and intestine (figure 2). A metabolic study with pigs fed a nickel supplemented diet was not performed because the metabolism cages were limited and also the stainless steel cages contained nickel. However, nickel has produced effects on growth rate and skin lesions in pigs similar to, but less dramatic and consistent, than those of cobalt, and it seems logical, on the basis of the present results, to ascribe this effect to increased zinc availability.

Soft tissues have been called “high priority” tissues for zinc by Rubin et al. (1961) and Miller (1973) since most soft tissues develop a greatly increased affinity for the available zinc when faced with a zinc shortage. The relative $^{65}$Zn concentration of the soft tissues such as the muscle has been reported to be greater for animals fed a zinc deficient diet than those fed a normal diet, but the reverse was true for bone (Heth and Hoekstra, 1966; Kienholz et al., 1965, Miller, 1969). In the present experiment, Ancona chicks fed the zinc sufficient diet showed markedly in-
EFFECT OF CO AND NI ON ZN AVAILABILITY IN CHICKS AND PIGS

Figure 2. The $^{65}$Zn concentration of the liver, kidney, intestine and muscle in male pigs (see text for calculation used). Bars indicate S.E.M.

Increased Zn concentration in bone and reduced Zn in liver and in muscle compared with chicks fed a basal (marginally zinc deficient) diet. The effect of zinc deficiency on $^{65}$Zn distribution in bone and soft tissues is in full agreement with this concept of conservation of available zinc in soft tissue. Supplementation of nickel or cobalt relatively increased Zn concentration in liver and bone, but slightly decreased concentration in muscle. The result indicated that cobalt and nickel improved zinc availability by showing increased $^{65}$Zn level in bone rather than muscle, which has lower priority than bone under conditions of zinc adequacy.

Supplemental cobalt improved zinc availability in pigs more than in chicks. The remarkable zinc availability in pigs more than cobalt may be explained by the difference in the absorption mechanisms of the two species which received the corn-soybean type diet high in calcium and which contained substantial amounts of phytate. The improved availability of zinc as a result of supplemental cobalt may be explained by the following two mechanism: (a) cobalt may preferentially precipitate in the intestinal lumen with calcium and phytate instead of zinc, thereby freeing zinc for absorption; (b) cobalt may activate intestinal phytase (E.C. 3.1.3.8) or substitute for zinc in phytase. The first mechanism in which cobalt may increase zinc absorption due to precipitation with calcium and phytate instead of zinc, was discussed in another paper. In the second mechanism, increased hydrolysis of phytate may result in decreased precipitation of zinc with phytate and calcium.

It has been suggested that phytase may be a non-specific alkaline phosphatase (E.C.3.1.3.1) in rat and chick intestine. Maddaiah et al., 1964; Davies and Motzok, 1972; Davies et al., 1970 have indicated that phytase was an isoenzyme of alkaline phosphatase in a series of experiments on chick intestine. They showed that alkaline phosphatase and phytase were close together on the elution curve from Sephadex G200 columns. This view has been disputed by Bitar and Reinhold (1972) who demonstrated that there were two distinct enzymes as assessed by differences in the two enzyme activities under various conditions of pH, substrate, inhibitor and cofactors. The enzymes were partially purified from the intestinal mucosa of rats, chicks and cows. However, these two enzymes showed broadly similar activity profiles after DEAE cellulose chromatography. Although it is difficult to confirm the suggestion without the full purification of both enzymes, the view that phytase is an isoenzyme of alkaline phosphatase was strengthened by Davies and Flett(1978), who demonstrated that the activity of phytase was similar to that of alkaline phosphatase under various conditions such as regional differences in the small intestine, cofactors for maximum enzyme activities, and zinc status of rats. The experiment also showed that zinc deficiency in rats markedly reduced intestinal phytase and alkaline phosphatase and resulted in decreased phytate disappearance from the ligated duodenal loops in situ. If phytase is an isoenzyme of alkaline phosphatase, there is abundant evidence that phytase activity varies with zinc status of an animal (Luecke et al., 1968, Williams, 1972). Other evidence showed that Co and Ni restored enzymatic activity to the inactive apoenzyme of alkaline phosphatase isolated from human placenta (Harkness, 1968). If the assumption that the phytase in an isoenzyme of the alkaline phosphatase is true, the observations suggest that Co or Ni
may activate phytase by substituting for zinc in the phytase molecule.

In previous experiments, supplemental cobalt or nickel induced increased serum and bone alkaline phosphatase activities in pigs but this may have been the result of increased zinc absorption. Although intestinal phytase and alkaline phosphatase were not measured in the experiments reported it is plausible that cobalt or nickel could increase the intestinal phytase activity during zinc deficiency. However, further studies are required to determine the mechanisms whereby cobalt or nickel increase zinc absorption.

**Literature Cited**


