SPARING EFFECTS OF COBALT AND NICKEL ON ZINC NUTRITION IN PIGS

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Summary

Three experiments were conducted to determine whether cobalt (Co) or nickel (Ni) could prevent zinc (Zn) deficiency signs in pigs fed a high calcium (Ca) corn-soybean diet. The basal diet contained 1.3% Ca, .93% phytic acid and means of 34 to 48 ppm Zn. After weanling, pigs in experiment 1 were fed the basal diet for 9 weeks, and was found that 50 ppm Co or Ni for 5 weeks increased average daily weight gain (ADG) and reversed skin lesions toward normal. These effects were similar to those of 100 ppm supplemental Zn. The Zn content and alkaline phosphatase activity of serum from pigs supplemented with Co or Ni were higher at 2 weeks and 4 weeks ($P < .05$) than those of the basal group. Zn content of bone, liver and kidney, and alkaline phosphatase activity in bone were increased after 5 weeks of supplementation with Co or Ni. In experiments 2 and 3, addition of 54 ppm and 27 ppm of either Co or Ni increased ($P < .05$) ADG and decreased incidence of skin lesions except in one group supplemented with 27 ppm Ni. Supplemental Co or Ni increased Zn in serum and alkaline phosphatase activity in serum and bone in both experiments. Over all experiments, supplemental Co or Ni decreased Zn deficiency signs in the following order of effectiveness: 54 ppm Co, 54 ppm Ni, 27 ppm Co and 27 ppm Ni. The alleviation of signs of Zn deficiency by Co or Ni may have been the result of increased availability of dietary Zn.

(**Key Words:** Zinc Nutrition Affected by Co and Ni)

Introduction

Studies of Zn metalloenzymes have shown that Co or Ni can substitute for Zn in numerous purified metalloenzymes *in vitro* (Vallee et al., 1958; Harkness, 1968; Lehky et al., 1973; Vallee, 1973). *In vivo*, Co can replace Zn in D-latic dehydrogenase of yeast (Curdel, 1966), alcohol dehydrogenase of yeast (Curdel and Iwatsuba, 1968) and RNA polymerase of E. coli (Speckhard et al., 1977).

Poor growth and severe skin lesions (parakeratosis) are the predominant gross signs of Zn deficiency in pigs (Tucker and Salmon, 1955). For the first time in 1969, Dahmer demonstrated that either Co or Zn stimulated growth and prevented skin lesions in weanling pigs fed a high Ca, corn-soybean type diet.

To confirm and expand on the observations of Dahmer (1969), three experiments were conducted with pigs to test whether Co and the closely related element Ni prevent Zn deficiency signs in pigs as measured by ADG, severity of skin lesions, and Zn concentration and alkaline phosphatase (EC 3.1.3.1) activity in blood serum, bone, liver, and kidney.

Materials and Methods

Three separate experiments were conducted with pigs from early weanling to nearly market weight. Eight pigs were assigned to each of eight dietary treatments in the first experiment and seven in the second and third, according to initial weight, litter, breed and sex. In the first experiment, Chester White, Poland China, Hampshire, and cross-bred weanling pigs averaging 16 kg were used. Chester White and Hampshire weanling pigs (13.6kg) were used for the second experi-

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Received January 21, 1988
Accepted July 26, 1988

ment, and Chester White and cross-bred weanling pigs (18.1 kg) in the third. All pigs received adequate Zn before being placed on experiment. They were housed in adjoining wooden pens with concrete floors and received diets *ad libitum* from wooden feeders and tap water *ad libitum* from iron troughs. The pigs apparently did not obtain significant quantities of Zn from the troughs as indicated by the severe Zn deficiency which developed in the groups on the basal diet.

The first experiment consisted of eight groups: 3 basal (to provide deficient animals for later recovery studies); basal + 1 ppm Ni; basal + 10 ppm Ni; basal + 10 ppm Co; basal + 50 ppm Co; and basal + 100 ppm Zn. After 9 weeks when pigs receiving 1 ppm Ni, 10 ppm Co and the basal diets started showing signs of zinc deficiency; at this time they were grouped and reassigned to the second stage (9-14weeks) following treatments according to previous treatments, and Zn deficiency signs such as growth rate, serum alkaline phosphatase activity and skin lesions. Pigs from two basal groups and the 1 ppm Ni group were reallotted in to basal, to a basal + 50 ppm Co and basal + 100 ppm Zn, and pigs from the other basal group and the 10 ppm Co group were reallotted to a basal and to a basal + 50 ppm Ni group. To evaluate the response from the initial treatments over a longer observation periods (because of satisfactory performance), the 10 ppm Ni, 50 ppm Co, and 100 ppm Zn groups were continued in the same treatments, previously. All treatment groups were continued for 5 weeks beyond the initial 9-week period.

The first experiment showed that 50 ppm Co or Ni was sufficient to prevent or alleviate Zn deficiency signs but 10 ppm Co or Ni was not. In the next two experiments Co and Ni were each supplemented at levels of 54 and 27 ppm, which were equimolar to 60 and 30 ppm of Zn, respectively. Thirty ppm of Zn added to the basal diet is borderline in preventing deficiency signs. The second and third experiments imposed seven treatments: basal; basal + 54 ppm Co; basal + 27 ppm Co; basal + 54 ppm Ni; basal + 27 ppm Ni; basal + 60 ppm Zn; and basal + 30 ppm Zn. The basal diet (table 1) contained 43 to 50 ppm Zn in the first experiment and 32 to 36 ppm Zn in the second and third experiments by chemical determination, and 1.3% Ca, .93% phytic acid and .66% phosphorus by calculation (NRC, 1968). Zinc, Co and Ni were added as carbonates in all but the first experiment, in which Zn was used as oxide.

TABLE 1. COMPOSITION OF BASAL DIET

Ingredient	%
Ground corn	68.6
Soybean meal	16.3
Dehydrated alfalfa meal	4.8
Dried brewer's yeast	3.0
Premix[1]	3.0
Mineral mix[2]	4.3
	100.0

[1] Antibiotic (chlorotetracycline), 22 mg/kg; vitamin D, 200 IU/kg; vitamin B_{12}, 15 µg/kg.
[2] Limestone, 22.3 g/kg; dicalcium phosphate, 15.6 g/kg; iodized salt, 5.0 g/kg.

Each pig was weighed and rated for skin lesions (Lewis et al., 1956) weekly by visual inspection. Feed consumption was recorded only for the third experiment. Blood for serum Zn and alkaline phosphatase determinations was collected at 8 weeks during the initial 9-week period and at 2 and 4 weeks after reallotment of pigs in the first experiment, and at 4 and 8 weeks in the other two experiments. The pigs were slaughtered at the end of the experiments at which time bone, liver, kidney and blood were taken for the determination of Zn and alkaline phosphatase except that blood was omitted in the first experiment. Samples were stored at −22°C until analyzed.

Tibia samples for Zn determination were extracted in a soxhlet extractor with diethyl ether for at least 24 hours. Diet, liver, kidney and the fat free tibia samples were put in crucibles and dried in vacuo at 80°C for 12-24 hours. The dried samples were dry ashed at temperatures not exceeding 600°C. The ashed samples were dissolved with gentle heating in 2 ml of distilled hydrochloric acid or nitric acid, and the resulting solution was quantitatively transferred and diluted with deionized water in a volumetric flask. All the glassware used for Zn determination was soaked in strong hydrochloric acid and rinsed with deionized water several times. Serum samples of the first experiment were wet ashed but in the second and third experiment were diluted with deionized water without ashing. Zinc was determined by atomic absorption spectrometry[4] according to the

TABLE 2. AVERAGE DAILY WEIGHT GAIN, SKIN RATING, SERUM ZN CONTENT AND SERUM ALKALINE PHOSPHATASE ACTIVITY (EXPERIMENT)[1,2]

Treatment	ADG (g/day)	1st phase — Initial 9 weeks		
		Skin rating[3]	Serum Zn at 8 weeks (μg Zn/ml serum)	Serum alkaline phosphatase at 8 weeks (μMolaes Pi/20 min/ml)
Basal 1	273 ± 45[e]	2.8 ± .5	.59 ± .03[e]	.92 ± .15[c]
Basal 2	278 ± 43[e]	3.0 ± .7	.49 + .03[e]	1.01 ± .13[e]
Basal 3	377 ± 48[de]	1.5 ± .6	.54 ± .03[e]	1.16 ± .20[e]
B + 10 ppm Co	396 ± 47[ce]	2.4 ± .8	.58 ± .03[e]	.97 ± .13[e]
B + 50 ppm Co	459 ± 51[cd]	0.0	.86 ± .10[d]	2.16 ± .29[d]
B + 1 ppm Ni	273 ± 57[e]	2.1 ± .5	.47 ± .02[e]	.92 ± .15[c]
B + 10 ppm Ni	483 ± 50[cd]	0.0	.50 + .13[e]	1.47 ± .27[e]
B + 100 ppm Zn	541 ± 35[c]	0.0	1.13 ± .06[c]	2.85 ± .38[c]

Treatment	ADG (g/day)	Skin rating	Final phase -- Last 5 weeks			
			2 weeks, serum		4 weeks, serum	
			Zn (μg Zn/ml)	Alkaline phosphatase (μ Moles Pi/ 20 min/ml)	Zn (μg Zn/ml)	Alkaline phosphatase (μ Moles Pi/ 20 min/ml)
Basal 4[b]	343 ± 83[f]	3.0 ± .7	.34 ± .03[e]	.97 ± .27[g]	.32 ± .03[g]	1.18 ± .37[c]
Basal 5[b]	398 ± 98[ef]	2.3 ± .7	.34 ± .02[e]	1.10 ± .26[fg]	.35 ± .03[g]	1.20 ± .27[e]
B + 50 ppm Co	747 ± 64[c]	1.4 ± .3	.49 ± .03[e]	1.79 ± .17[de]	.53 ± .05ef	2.22 + .31[cd]
B + 50 ppm Ni	757 ± 58[e]	.9 ± .4	.78 ± .11[d]	1.94 ± .13[d]	.67 ± .06[de]	2.89 ± .31[c]
B + 100 ppm Zn	640 ± 38[cd]	1.0 ± .3	.96 ± .06[c]	2.78 ± .28[c]	.71 ± .04[d]	3.06 ± .58[c]
B + 50 ppm Co	572 ± 47	0.0	.74 ± .06[d]	1.83 ± .22[d]	.59 ± .06[dc]	2.28 ± .31[cd]
B + 10 ppm Ni	505 ± 90[df]	0.0	.48 ± .04[f]	1.43 ± .28[df]	.43 ± .04[fg]	1.48 ± .28[de]
B + 100 ppm Zn	729 ± 37[c]	0.0	1.07 ± .05[c]	2.91 ± .24[c]	.86 ± .06[c]	2.88 ± .22[c]

[1] Mean ± SEM.
[2] Means within a column with different superscripts differ ($P < .05$).
[3] 0 is normal, 5 is severely parakeratotic.
[4] Groups reallotted at 9 weeks.

standard procedure for the instrument.

The alkaline phosphatase activity was measured by a modification of the method of Bessey et al. (1946). The assay medium consisted of one volume of 1.5×10^{-2} M disodium p-nitrophenyl phosphate[5] as substrate and one volume of buffer containing 1.5 M 2-amino-2-methyl-1-propanol and 3×10^{-3} M $MgCl_2$ adjusted to pH 10.3 with HCl. The reaction system contained 1 ml each of buffer solution, substrate, and diluted blood serum or tissue homogenized with a Polytron[6]. Following 20 minutes incubation at 37°C, the reaction was stopped by the addition of 1 ml of 1 N NaOH, and the p-nitrophenol was determined at 400 nm in a spectrophotometer. Five mm chick center sections of bone were prepared by cutting the tibias longitudinally; Zn was measured in the shaft position and alkaline phosphatase activity was determined in the epiphyseal plate portion.

[4] Atomic absorption spectrophotomer, Model 403, Perkin Elmer, Corp., Norwalk, CT.
[5] Sigma, St. Louis, Mo.
[6] Brinkmann Instruments, Westburg, NY.

After kidney samples were homogenized by a Potter-Elvehjem homogenizer, leucine aminopeptidase (EC 3.4.1.2) activity in kidney was measured in the third experiment by the method of Wachsmuth et al. (1966) using L-leucine-p-nitroanilide as substrate at 37°C and pH 7.0. The data were analyzed statistically by Duncan's (1955) multiple range test as modified by Kramer (1956).

Results

Experiment 1.

During the initial 9-week period, supplementation with 10 ppm Ni or 50 ppm Co or 100 ppm Zn improved ($P < .05$) weight gain and resulted in no skin lesions (table 2). While the serum Zn and alkaline phosphatase activity of the 10 ppm Ni supplemented group was not different from that of the basal groups at 8 weeks, the supplementation of 50 ppm Co increased Zn concentration and alkaline phosphatase activity in serum. Weight gain, skin rating, serum Zn, and serum alkaline phosphatase values of the 10 ppm Co and 1 ppm Ni groups were not different from those of the basal groups. The supplementation of 100 ppm Zn produced the highest weight gain, and Zn concentration and alkaline phosphatase activity in serum among the treatments. The weekly skin rating was not included in the table; however, skin lesions appeared in the basal groups at 3 weeks and became progressively worse until 9 weeks (the end of initial period) with an average score of 2.4 (0 is normal and 5 is very severe). Skin lesions started from soft skin such as deep thigh, behind of ear lobes and eyelids, and progressed to belley and legs.

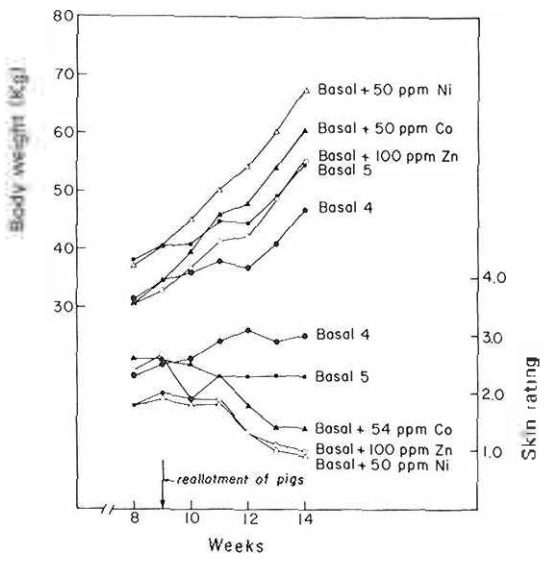

Figure 1. Body weight and skin ratings before and after pigs were reallotted. (Experiment 1)

During the later 5-week period (after the reallotment of pigs), a supplementation of 50 ppm Co or Ni to Zn-deficient pigs resulted in improved weight gain and partially healed skin lesions (table 2). This was similar to the effect of the 100 ppm supplemental Zn, whereas the two newly created basal groups showed slow weight gain and developed more severe skin lesions during the latter 5-week phase (figure 1). Zinc and alkaline phosphatase activity in the serum of the 50 ppm Co and Ni groups increased within 2 weeks and significantly higher ($P < .05$) at 4 weeks.

Supplementation with 50 ppm Co or Ni (after the reallotment of pigs) showed a tendency toward increased Zn in bone, liver and kidney (table 3). The bone alkaline phosphatase activity of these 50 ppm Co and Ni groups was higher ($P < .05$) than that of the basal groups and similar to that of the 100 ppm Zn supplemented group, after the reallotment of pigs. Supplementation of 50 ppm Co at the beginning of this experiment produced higher ($P < .05$) levels of Zn in bone, liver and kidney and of alkaline phosphatase in bone, whereas the Zn content and the enzyme activity in these tissues of the 10 ppm Ni supplemented group were similar to those of the basal groups.

Although the weight gain of the 10 ppm Ni group was similar to that of the 50 ppm Co group for the initial 9 weeks, the group fed the 50 ppm Co supplemented diet from the beginning of the experiment gained faster during the later 5 weeks than did the Ni group and had elevated Zn and increased alkaline phosphatase in serum and bone throughout the experiment, indicating that 50 ppm Co was superior to 10 ppm Ni. During the entire experimental period, supplementation of 100 ppm Zn yielded the largest weight gain, the highest Zn in serum, bone, liver and kidney, and the highest alkaline phosphatase in serum and bone. The main findings were that supplementation of 50 ppm Co to weanling pigs for the entire experimental period or 50 ppm of supplemental

TABLE 3. ZN CONTENT AND ALKALINE PHOSPHATASE IN BONE, LIVER AND KIDNEY (EXPERIMENT 1)

Treatment	Bone		Liver	Kidney	
	Zn (μg/g FFB)[a,d]	Alkaline phosphatase (μ Moles Pi/20 min/g wet bone)[d]	Zn (μg/g DM)[b,d]	(μg/g DM)[d]	Alkaline phosphatase (μ Moles Pi/20 min/g)[d]
Basal 4[c]	44 ± 5[hj]	58 ± 12[f]	95 ± 5[h]	86 ± 4[g]	347 ± 97[e]
Basal 5[c]	40 ± 3[ij]	58 ± 12[f]	95 ± 5[h]	86 ± 1[g]	432 ± 59[e]
B + 50 ppm Co[c]	52 ± 2[h]	93 ± 11[e]	116 ± 5[gh]	93 ± 1[eg]	408 ± 42[e]
B + 50 ppm Ni[c]	46 ± 2[hi]	121 ± 13[e]	116 ± 6[gh]	90 ± 6[fg]	410 ± 75[e]
B + 100 ppm Zn[c]	81 ± 2[f]	124 ± 13[e]	160 ± 11[f]	106 ± 3[e]	273 ± 42[e]
B + 50 ppm Co	63 ± 4[g]	92 ± 7[e]	131 ± 6[g]	101 ± 8[ef]	248 ± 29[e]
B + 10 ppm Ni	35 ± 3[j]	54 ± 11[f]	115 ± 9[gh]	86 ± 2[g]	370 ± 52[e]
B + 100 ppm Zn	115 ± 5[e]	100 ± 8[e]	207 ± 17[e]	103 ± 4[e]	270 ± 25[e]

[a]FFB Means fat free bone.
[b]DM means dry matter.
[c]Groups reallotted at 9 weeks.
[d]Mean ± SEM.
[e-j]Means within a column with different superscripts differ ($P < .05$).

Co or Ni to Zn-deficient pigs for the last 5 weeks prevented or alleviated Zn deficiency signs. These effects approached those observed from 100 ppm supplemental Zn as measured by growth rate, the prevention of healing of skin lesions, Zn content of serum, liver and kidney, and alkaline phosphatase activity in serum and bone.

Experiments 2 and 3.

These two experiments were designed to compare the effectiveness of two levels (54 ppm and 27 ppm) of supplemental Co or Ni with the molar equivalents of 60 ppm and 30 ppm of supplemental Zn in preventing Zn deficiency signs in growing swine. Supplementation with 54 ppm or 27 ppm of either Co or Ni in both experiments promoted higher ($P < .05$) ADG, lower incidence of skin lesions and better feed efficiency (only experiment 3) than in the basal group (table 4). The ADG of the 27 ppm Ni group in the second experiment was the only exception. Furthermore, the 54 ppm Co or Ni groups had higher ADG ($P < .05$) and lower incidence of skin lesions in both experiments, and better feed efficiency in the third experiment, than the groups given 27 ppm Co or Ni. A group fed 27 ppm Co in the second experiment was the exception. The ADG and skin lesions of 54 ppm Co groups in both experiments and of the 54 ppm Ni and 27 ppm Co groups in the second experiment were similar to those of the groups supplemented with 60 and 30 ppm Zn, whereas growth rate and skin condition of 54 ppm Ni, 27 ppm Co and 27 ppm Ni groups in the third experiment were inferior to those of both groups supplemented with Zn. In general, pigs receiving 27 ppm Co were superior to those receiving the same level of Ni.

Serum Zn concentration of groups supplemented with 54 ppm and 27 ppm of either Co or Ni in both experiments tended to increase above basal valued at 4 weeks and 8 weeks (table 5). Furthermore, serum Zn level of the 54 ppm Co group in the third experiment was higher ($P < .05$) than the basal value at 8 weeks. At the end of both experiments, supplementation of 54 ppm or 27 ppm of either Co or Ni yielded higher ($P < .05$) serum Zn concentrations in all except the 27 ppm Ni group in the second experiment. Supplementation with 30 ppm Zn resulted in increased Zn concentration in serum, bone and liver over the groups supplemented with either level of Ni or Co, but Zn concentration in these tissues of the 30 ppm Zn groups was generally lower than in the 60 ppm Zn groups. Supplementation of 60 ppm Zn promoted the highest Zn concentration in the kidney; in this respect all the

TABLE 4. GROWTH RATE, SKIN RATINGS AND FEED EFFICIENCY (EXPERIMENTS 2 AND 3)

			+Co		+Ni		+Zn	
	Exp	Basal	54 ppm	27 ppm	54 ppm	27 ppm	60 ppm	30 ppm
ADG (g/day)[a]	2	486 ± 62[d]	658 ± 33[bc]	683 ± 17[b]	705 ± 43[b]	544 ± 37[cd]	729 ± 58[b]	742 ± 41[b]
	3	190 ± 31[f]	702 ± 21[bc]	351 ± 72[e]	591 ± 48[d]	342 ± 89[e]	687 ± 12[c]	731 ± 18[b]
Skin rating[a,g]	2	1.9 (6/7)	.2 (1/8)	0.0 (0/8)	0.0 (0/8)	1.4 (4/7)	0.0 (0/8)	0.0 (0/8)
	3	3.3 (8/8)	0.0 (0/8)	2.0 (7/8)	0.5 (2/8)	2.8 (7/8)	0.0 (0/8)	0.0 (0/8)
Feed efficiency (feed/gain)[a]	3	6.09	3.30	3.64	3.39	4.23	3.32	3.19

[a]Mean ± SEM, or mean alone when SEM could not be calculated.
[b-f]Means within a row with different superscripts differ ($P < .05$).
[g]Numbers in parentheses show the fraction of pigs having skin lesions.

TABLE 5. ZN CONTENT OF SERUM, BONE, LIVER, AND KIDNEY, (EXPERIMENTS 2 AND 3)

			+ Co		+ Ni		+ Zn	
Organ	Exp	Basal	54 ppm	27 ppm	54 ppm	27 ppm	60 ppm	30 ppm
Serum(μg/ml)[c]								
4 weeks	2	.24±.01[d]	.42±.09[d]	.35±.04[d]	.31±.03[d]	.44±.05[d]	.47±.05[d]	.44±.04[d]
	3	.24±.03[e]	.36±.04[e]	.26±.03[e]	.33±.04[e]	.33±.03[e]	.61±.05[d]	.60±.06[d]
8 weeks	2	.24±.02[e]	.32±.06[e]	.32±.03[e]	.27±.02[e]	.32±.05[e]	.69±.04[d]	.50±.02[d]
	3	.21±.05[f]	.40±.04[c]	.26±.03[f]	.30±.03[ef]	.23±.02[f]	.71±.07[d]	.49±.07[e]
Slaughter	2	.21±.02[g]	.32±.03[f]	.49±.08[e]	.37±.05[f]	.27±.06[fg]	.69±.04[d]	.56±.08[de]
time	3	.14±.02[f]	.33±.02[e]	.26±.02[e]	.30±.02[e]	.26±.01[e]	.64±.03[d]	.56±.04[d]
Bone	2	27.0±1.6[g]	27.0±5.3[fg]	45.5±5.5[ef]	37.1±2.8[fg]	29.7±2.1[fg]	89.9±5.5[d]	57.3±3.9[e]
(μg/FFB)[a,c]	3	31.4±1.9[ef]	38.1±4.2[e]	25.8±1.8[f]	26.1±2.0[f]	30.0±2.8[ef]	84.5±5.9[d]	52.7±3.0[d]
Liver	2	91.4±7.7[e]	96.3±3.4[e]	95.3±4.2[e]	103.0±2.2[e]	92.3±3.8[e]	119.9±8.2[d]	108.3±7.7[d]
(μg/g DM)[b,c]	3	86.2±3.8[ef]	96.6±6.7[ef]	83.3±4.0[f]	93.4±5.4[e f]	84.6±5.0[f]	141.4±17.2[d]	111.3±5.7[e]
Kidney (μg/g DM)[c]	3	84.4±2.6[e]	85.1±1.8[e]	80.7±2.2[e]	84.7±1.4[e]	82.4±1.4[e]	94.2±2.7[e]	87.0±1.2[e]

[a]FFB means fat free bone.
[b]DM means dry matter.
[c]Mean ± SEM.
[d-g]Means within a row with different superscripts differ ($P < .05$).

other treatment groups were similar to one another.

In both experiments, 54 ppm Co or Ni increased serum alkaline phosphatase activity at 4 weeks as compared to the basal value (table 6). However, only the increase in the 54 ppm Co group was significant ($P < .05$). The 54 ppm Co or Ni supplemented groups also had higher ($P < .05$) serum alkaline phosphatase activity at 8 weeks and at the end of the experiments. Supplementation with 27 ppm Co or Ni appeared to increase the serum alkaline phosphatase activity over that of the basal group at 8 weeks and at the end of both experiments, but the difference was significant ($P < .05$) only for the 27 ppm Co groups. The alkaline phosphatase activity in bone and kidney was higher ($P < .05$) for 54 ppm Co or Ni groups than for the basal, but the apparent increase for the 54 ppm group in the second experiment was not significant. Supplementation of 27 ppm Co resulted in increased ($P < .05$) alkaline phosphatase activity in bone and kidney in both

TABLE 6. ALKALINE PHOSPHATASE OF SERUM, BONE, LIVER, AND KIDNEY AND AMINOPEPTIDASE OF KIDNEY, EXPERIMENTS 2 AND 3

		Alkaline phosphatase (μ Moles Pi/20 min/g tissue or ml serum)						
			+Co		+Ni		+Zn	
	Exp	Basal	54 ppm	27 ppm	54 ppm	27 ppm	60 ppm	30 ppm
Serum[a]								
4 weeks	2	1.42±.13^{d}	2.16±.28^{bd}	1.72±.30^{cd}	2.26±.44^{bd}	1.68±.23^{cd}	3.08±.50^{b}	2.81±.27^{bc}
	3	1.22±.10^{f}	2.76±.33^{cd}	1.47±.19^{ef}	2.12±.24de	1.76±.26^{ef}	3.88±.38^{b}	3.48±.40^{bc}
8 weeks	2	1.18±.08^{d}	2.04±.24^{c}	1.85±.09^{c}	1.76±.12^{c}	1.60±.13^{cd}	3.21±.38^{b}	2.81±.15^{b}
	3	.93±.06^{d}	2.74±.26^{b}	1.44±.18^{cd}	1.78±.10^{c}	1.09±.18^{cd}	3.34±.39^{b}	2.79±.26^{b}
Slaughter	2	1.33±.11^{c}	1.86±.12^{df}	1.94±.10^{cd}	2.11±.11^{c}	1.63±.22de	2.78±.19^{b}	2.64±.14^{b}
time	3	.86±.12^{e}	2.00±.21^{c}	1.35±.11^{ce}	1.55±1.08^{cd}	1.13±.15de	3.00±.40^{b}	2.77±.28^{b}
Bone[a]	2	213±14^{d}	230±20^{d}	318±22^{bc}	331±36^{bc}	252±25^{bd}	381±36^{b}	399±34^{b}
	3	130±16^{d}	176±22^{c}	173±13^{c}	176±17^{c}	148±16^{bd}	210±13^{b}	215±22^{b}
Liver[a]	2	27.9±3.4^{c}	31.7±2.7^{c}	38.5±1.5^{c}	32.6±3.2^{c}	33±4.7^{c}	42.4±3.3^{b}	35±3.9^{b}
	3	22.3±2.3^{c}	20.5±1.9^{c}	23.9±3.9^{c}	23.9±3.9^{c}	24.6±3.2^{c}	31.4±6.4^{b}	23.9±3.2^{c}
Kidney[a]	3	395±38^{d}	499±29^{ac}	515±48^{b}	489±32^{c}	488±49^{bc}	515±31^{b}	490±20^{bc}
		Aminopeptidase (μ Moles leucine/min/g tissue)						
Kidney[a]	3	7.79±.88^{b}	7.91±.45^{b}	8.26±.50^{b}	8.01±.78^{b}	8.03±.63^{b}	8.13±.57^{b}	8.16±59^{b}

[a]Mean ± SEM.
[b-f]Means within a row with different superscripts differ ($P < .05$).

experiments. The 27 ppm Ni group also had increased alkaline phosphatase activity in bone and kidney, but the difference was significant only in the kidney. Supplementation of 60 ppm Zn yielded the highest activity of alkaline phosphatase in serum, bone, liver and kidney, and 30 ppm Zn yielded the second highest. The differences due to level of Zn were not significant except for alkaline phosphatase activity in liver of the third experiment. Neither was there any difference in the activity of aminopeptidase in kidney among treatments, including the basal group.

The basal group of the third experiment showed the earliest skin lesions at 4 weeks. The lesions peaked (3.9) at 6 weeks and remained about the same throughout the experiment (figure 2). The weight gain of the basal group declined at 4 weeks, increased slowly until 6 weeks, and plateaued thereafter. Weight gain was inversely related to the rating of skin lesions in the basal group. Supplemental Co or Ni reduced the incidence and severity of skin lesions in decreasing order: 54 ppm Co; 54 ppm Ni; 27 ppm Co, and 27 ppm Ni. Weight gains of the treated groups ranked in reverse order from skin ratings.

Overall, the zinc concentrations in serum and alkaline phosphatase activities in serum and bone of supplemented groups were in similar order to ADG and in almost reversed order from skin ratings. Although one pig receiving 54 ppm Co did show minor skin lesions, this group had skin condition and ADG similar to those of the Zn supplemented groups. These findings showed that Co, and to a lesser extent Ni, were effective in preventing or greatly reducing the severity of Zn deficiency signs.

Discussion

The marked increase in ADG, the reduced incidence of skin lesions and the improved feed efficiency showed that Co and Ni were able to prevent or nearly prevent the outward signs of Zn deficiency in weanling and growing pigs fed a high Ca corn-soybean diet containing level of 1.3%. These results were similar to the effect of supplemental Co in pigs shown by Dahmer(1969).

Furthermore, like supplemental Zn, supplemental Co or Ni was therapeutically effective in Zn-deficient growing pigs and indicated by increased growth rate and healed skin lesions (figure 1).

The increased weight gain of pigs fed the Co or Ni supplemented diets might be explained by increased feed consumption and, therefore, greater Zn intake. In the third experiment, the supplemented groups were at least twice as efficient in utilizing feed as the basal, while skin lesions first appeared in the basal group at 4 weeks and the growth rate of the group was reduced markedly at the same time(figure 2). A similar relationship between the incidence of skin lesions and the reduced growth rate was observed in experiments 1 and 2. The investigation by Miller et al.(1968) demonstrated that Zn deficiency reduced the growth rate of baby pigs before feed intake was affected. On the other hand, supplemental Zn and Ni may increase feed intake, as shown by Henkin and Bradley (1970), by correcting hypogeusia, loss of taste. Cobalt, chemically similar to Zn and Ni, could be expected to have the same effect. Although paired feeding was not employed and Zn concentrations of saliva, as a possible index of taste perception, were not determined in the present experiments, the observations suggest that the supplemental Co or Ni affected growth rate and skin lesions more than could be accounted for by increased feed intake.

Zinc concentration in bone, liver and serum could give an indication of the Zn status of animals, since Zn in these tissues has been shown to decrease with Zn deficiency in pigs (Hoekstra et al., 1956; Miller et al., 1968), rats (Kirchgessner and Pallauf, 1972a) and ruminants (Miller, 1969). The supplementation of 54 ppm Co or Ni resulted in increased Zn concentrations in serum. The supplemental groups also tended to have increased Zn concentrations in liver and bone. The Zn content of serum, bone and liver declined in response to depletion and increased upon repletion of Zn in growing rats (Kirchgessner and Pallauf, 1972a; Kirchgessner and Pallauf, 1972b). The latter results were similar to those found in the present studies from supplementing Zn-deficient pigs with either 50 ppm Co, 50 ppm Ni or 100 ppm Zn. This observation suggests that supplemental Co or Ni resulted in improved availability of Zn in pigs fed a corn-soybean type diet high in calcium.

The activity of alkaline phosphatase decrease in

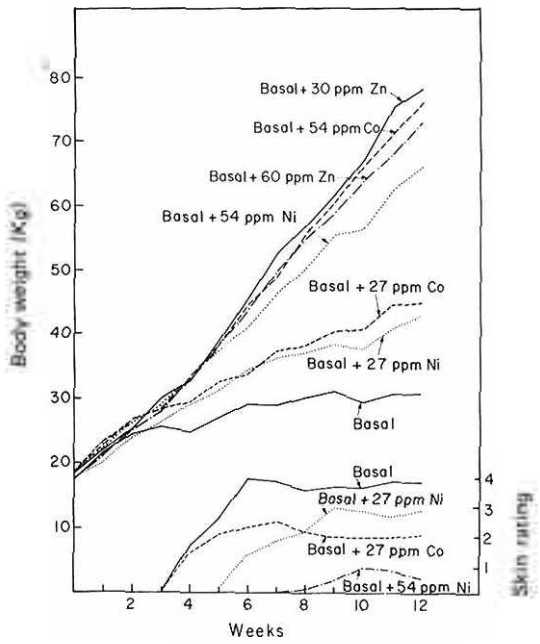

Figure 2. Growth rate and skin ratings. Skin ratings for the groups supplemented with 54 ppm Co, 30 ppm Zn, and 60 ppm Zn are not shown as they remained along base line. (Experiment 3)

serum, bone and other organs of Zn-deficient rats, pigs, dairy cows and chicks, as was well documented in a review paper by Kirchgessner et al. (1976). The supplementation of 54 ppm Co or Ni began to increase alkaline phosphatase activity over the basal value at 4 weeks, and the differences in enzyme activity were significant at 8 weeks and at the end of experiments 2 and 3. The increased alkaline phosphatase in the serum of the treated groups seems to be closely related to increased Zn concentration in serum throughout all the pig experiments, including those with the Zn supplemented groups. In experiment 1, the supplementation of 50 ppm Co or 50 ppm Ni to Zn-deficient pigs yielded increased activity of serum alkaline phosphatase within 2 weeks. This was in agreement with the review of Kirchgessner et al. (1976), who reported that the alkaline phosphatase in serum was rapidly changed in Zn-deficient rats fed a Zn sufficient diet.

The alkaline phosphatase activity in the bone of the Co or Ni supplemented groups did not seem to be closely related to the Zn content in bone

and this is similar to the result of Dahmer(1969). It is possible that supplementation with Co or Ni increased the Zn content of the epiphyseal plate regions of the bone but to a lesser extent that of the shaft region, since Zn was measured in the shaft portion and the enzyme activity was measured in the epiphyseal plate region. On the other hand, Co or Ni may have directly increased the alkaline phosphatase activity of bone by substituting for Zn. Bone is probably the largest contributor of alkaline phosphatase to serum as suggested by Lin and Hoekstra (1969). Cobalt restored alkaline phosphatase activity *in vitro* to the inactive apoenzyme from *E. coli* (Plocke and Vallee, 1962; Simpson and Vallee, 1968) and from human placenta (Harkness, 1968). Cobalt and Ni have also been found restore or activate alkaline phosphatase as well as several Zn metalloenzymes *in vitro* (Vallee et al., 1968; Lehky et al., 1973; Kwan et al., 1975; Strover et al., 1976; Sugiura et al., 1976). Furthermore, Co can substitute for Zn in its enzymes *in vivo* in microorganisms grown in Zn-deficient and Co-enriched media: lactic dehydrogenase (Curdel, 1966), alcohol dehydrogenase (Curdel and Iwatsuba, 1968) and RNA polymerase (Speckhard et al., 1977). The increase in the activity of alkaline phosphatase in bone and serum of the Co or Ni supplemental groups in the present studies could be explained by such an activating effect of Co or Ni. This possibility is being discussed in more detail in another paper.[7]

It has been shown that phytic acid and Ca reduce the availability of Zn (Oberleas, 1966; Bafundo et al., 1984) and that Ca reduces the absorption of Zn (Heth et al., 1966). Vohra et al. (1965) demonstrated that phytic acid binds several metals in the following order: Cu^{++}, Zn^{++}, Ni^{++}, Co^{++}, Mn^{++}, Fe^{++} and Ca^{++} at pH 7.4. This was similar to the observation of Maddainah et al. (1964). Furthermore, phytate in the presence of Ca precipitates Zn from a solution at physiological pH as shown in a review paper by Oberleas, 1973. Therefore, less Zn would be expected to be available at the absorption site in the presence of phytate and Ca. Our basal diet contained high Ca (1.3%) and substantial phytate (.93%). Pigs fed the basal diet showed severe Zn deficiency even though the diets contained marginally deficient Zn; 48 ppm in the first experiment and 34 ppm Zn in the second and third experiments. These observations were similar to those of Davies *et al.*, 1985. They showed that increasing dietary Ca in the presence of phytate significantly reduced growth rate in rats and supplementation of Zn increased growth rate and plasma Zn concentration. Supplemental Co or Ni seemed to improve Zn availability as indicated by elevated Zn levels in serum and sometimes in other tissues. It is possible that Co or Ni precipitated with phytate-Ca complexes instead of Zn so that more Zn was available for absorption. Further study is required to test this possibility.

The sparing effects of Co or Ni on Zn nutrition shown in this paper for swine could not be demonstrated in rats fed a semipurified diet (unpublished data, A. S. Chung, Kirchgessner and Pallauf, 1973; Spears et al., 1978) nor in chicks (unpublished data, W. G. Hoekstra). However, the sparing effects of Co or Ni on Zn were shown in chicks fed a practical type, corn-soybean diet high in calcium as measured by increased weight gain and reduced feather defects.

These findings suggest that the sparing effects of Co or Ni are depended upon diet rather than species. Although increased feed intake or substitution for Zn in alkaline phosphatase or other Zn-dependent enzymes may explain the effects of supplemental Co or Ni, the most likely explanation for the sparing effects of Co or Ni on Zn would appear to be the result of increased availability of dietary Zn in pigs fed a corn-soybean type diet high in calcium.[8]

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