SPARING EFFECTS OF COBALT OR NICKEL ON ZINC NUTRITION AND THE DIFFERENCE IN ZINC ABSORPTION BETWEEN ANCONA AND NEW HAMPSHIRE X LEGHORN CROSS CHICKS

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Summary

Experiments were conducted to determine whether supplemental cobalt (Co) or nickel (Ni) would prevent the signs of zinc (Zn) deficiency in chicks fed a high calcium (1.5%) corn-soybean diet and to examine the difference in ^{65}Zn absorption rates between inbred Ancona and crossbred New Hampshire X Single Comb White Leghorn chicks. In the initial experiment, the supplementation of 27 ppm Ni, 27 ppm Co or 54 ppm Co to a basal diet increased weight gain and reduced feather defects; 54 ppm Ni tended to increase weight gain but did not reduce feather defects. In further experiments, chicks fed the diet supplemented with 54 ppm Co usually showed increased weight gain and reduced feather defects; however, chicks fed a diet supplemented with 54 ppm Ni less frequently showed these effects. In another test, Ancona chicks fed a diet supplemented with 30 ppm Zn (except during a ^{65}Zn absorption study period) showed lower weight gain, more feather defects and less ^{65}Zn absorption than did New Hampshire X Leghorn cross chicks. Similar results were achieved with two strains of chicks fed the basal and 54 ppm Ni, 54 ppm Co or 60 ppm Zn supplemented diets. The sparing effects of Co on Zn which were commonly observed and the lesser effect of Ni on Zn were shown to be, at least in part, the result of increased availability of dietary Zn. That Ancona chicks required more Zn than New Hampshire cross chicks for the development of feathers and for growth is partly the result of decreased Zn absorption from the type of diets fed.

(Key Words: Co and Ni on Zn Nutrition)

Introduction

Sparing effects of Co or Ni on Zn nutrition have been shown with pigs fed a practical corn-soybean diet.[3] Supplementation of Co or Ni to the basal diet improved weight gain and reduced skin lesions (parakeratosis), and also increased Zn in serum and alkaline phosphatase activity in serum and bone. However, Co or Ni did not have a sparing effect when chicks and rats were fed a semipurified diet (W.G. Hoekstra and A. S. Chung, unpublished data). In another study, an inbred line of Ancona chicks required a higher level of Zn than some inbred Leghorn lines to prevent a frayed feather condition (Englert et al., 1966). Later, Sunde et al. (1970) showed that the incidence of fraying in inbred Anconas was increased by genetic selection for this abnormality. The Zn requirement of these selected Anconas was higher than that of the commercial egg-laying strain chicks. The present study was designed to test whether Co and Ni have sparing effects on Zn in both Ancona and New Hampshire X Leghorn cross chicks. Further studies were conducted, using whole body ^{65}Zn assay technique (Heth and Hoekstra, 1965), to examine whether the difference of Zn requirement between the two strains was derived from a difference in ^{65}Zn absorption and to measure the effect of supplemental Co or Ni.

Materials and Methods

Five experiments were conducted with a day-old chick assigned randomly to treatments without segregation by sex and caged in a stainless steel

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battery with brooder temperatures ranging from 40°C at the start to 32°C at the end of the experiments. The experimental period was from 3 to 5 weeks. A special inbred line of Ancona[4] chicks was used in experiments 1 and 2; New Hampshire X Single Comb White Leghorn[4] chicks were used in experiment 3; and both strains of chicks were used in experiments 4 and 5. Feed and distilled water were supplied *ad libitum* in aluminum and stainless steel troughs, respectively. The basal diet was a high Ca corn-soybean type diet (table 1), which contained 33 to 43 ppm Zn (chemical determination) and 1.5% Ca, 0.7% P, 1.19% phytic acid and 1.43% arginine according to Tables (NRC, 1971).

TABLE 1. BASAL DIET

Ingredients	g/kg
Ground corn	551.4
Soybean meal	350.0
Dehydrated alfalfa meal	50.0
Mineral mix[1]	42.1
Vitamin mix[2]	5.0
DL-methionine	1.5

[1]$CaCO_3$ 22.5 g/kg; $CaHPO_4$ 14.3 g/kg; $MnSO_4 \cdot H_2O$ 0.3 g/kg; iodized salt 5.0 g/kg.

[2]Vitamin A 5000 IU/kg; vitamin D_3 1000 IU/kg; riboflavin 3 mg/kg; pantothenic acid 5 mg/kg; niacin 10 mg/kg; choline chloride 500 mg/kg; vitamin B_{12} 10 μg/kg diluted in casein.

Experiment 1 was designed to study the effects of 27 ppm or 54 ppm Ni, 27 ppm or 54 ppm Co, and 30 ppm or 60 ppm Zn added to the basal diet. Growth rate and feather defects were used as the criteria of Zn deficiency. The 27 ppm and 54 ppm Ni and Co were in the same molar ratio as 30 and 60 ppm Zn.

In experiment 2, 54 ppm of Ni, of Co or of both Ni and Co were added to the basal and the Zn sufficient (+60 ppm Zn) diets to asertain whether Ni, Co, or Ni plus Co was effective in preventing Zn dificiency, and whether Co or Ni had additive effects in a Zn sufficient diet with Ancona chicks. Experiment 3 was designed the same as experiment 2 except that New Hampshire X Leghorn cross chicks were used.

Experiment 4 was designed to compare the absorption of ^{65}Zn in Ancona and New Hampshire X Leghorn cross chicks. A diet marginal in Zn (+30 ppm Zn) was fed to chicks except during a 2-1/2 day ^{65}Zn absorption study period. Ancona chicks were fed the test diets (Ni, Co or Zn supplemental diet) for 1-1/2 days before and for 1 day after ^{65}Zn administration, and New Hampshire X Leghorn cross chicks were fed test diets (basal diet and Ni or Co supplemented diets) for 1 day before and 1-1/2 days after ^{65}Zn administration. The diet marginal in Zn was fed to Ancona chicks for 9-1/2 days and to New Hampshire X Leghorn cross chicks for 10-1/2 days before the ^{65}Zn administration period and for 9 days for Ancona and 8 days for New Hamshire X Leghorn chicks after the ^{65}Zn administration period.

The ^{65}Zn absorption studies of experiment 4 were designed according to the method of Heth and Hoekstra (1965). After a 12-hour fast, 10 chicks from each treatment group were fed ^{65}Zn mixed with the appropriate diet (table 5) for a 1-1/2 hour period. This length of feeding was chosen because the first dye was excreted 1 hour and 45 minutes after an oral administration of Carmine-aluminum lake[5] (about 50 mg) in gelatin capsules.[6] Two μCi $^{65}ZnCl_2$[7] were mixed well with 3 g of their appropriate diet. Five additional chicks from the same dietary group received, intramuscularly into the right thigh, 1 μCi ^{65}Zn in the form of Zn:glycine (1:4) complex in water, pH 7.4 Total body ^{65}Zn activity was measured in a whole animal gamma scintillation counter every 4 or 5 hours initially, then every 12 hours to 3 days post ^{65}Zn administration, and every 24 or 36 hours thereafter until 9 or 10 days after ^{65}Zn administration, when the chicks were killed by decapitation.

In experiment 5 both Ancona and New Hampshire X Leghorn cross chicks were started at one day of age and fed the diets in table 6. This experiment was also designed to compare the two genetic groups for Zn absorption. ^{65}Zn absorption

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studies of this experiment were similar to those of experiment 4 except that fasting was 11-1/2 hours and 3 μCi $^{65}ZnCl_2$ were mixed with 4 g of the appropriate diet for the oral administration of ^{65}Zn.

The chicks were weighted and observed for feather defects at the end of the experiments. Feather scores were based on "frayed and broken" feathers by visual inspection and rated from 0 for normal to 5 for most severe defects (Nielsen et al., 1968). Nickel, Co and Zn were added as carbonates.

Diet samples were ashed overnight in a muffle furnace at temperatures not exceeding 580°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 waster in a volumetric flask. Zinc was determined by atomic absorption spectrometry[8] according to standard procedure for the instrument. The data were analyzed statistically using Duncan's multiple range test as modified by Kramer and using student's t-test (Steel and Torrie, 1960). The feather score differences were not tested statistically since visual inspection could yield only integer values.

TABLE 2. EFFECT OF NICKEL, COBALT AND ZINC ON WEIGHT GAINS AND FEATHERING OF ANCONA CHICKS (EXPERIMENT 1)

Additions to basal, ppm	Weight gain (g)[1] for 35 days	Feather score[1,3]
0	169 ± 5[e2]	3.6 ± .4
27 Ni	204 ± 13[b]	2.6 ± .5
54 Ni	197 ± 8[de]	4.0 ± .4
27 Co	216 ± 10[cd]	3.2 ± .4
54 Co	233 ± 9[bc]	2.3 ± .4
30 Zn	271 ± 11[a]	1.1 ± .4
60 Zn	255 ± 15[ab]	.6 ± .4

[1] Mean ± SEM.
[2a-e] Means within a column with different letter are significantly different ($P < .05$).
[3] 0 = normal; 5 = serious fraying.

TABLE 3. PERFORMANCE OF ANCONA CHICKS FED Ni, Co OR Zn ADDITIONS (EXPERIMENT 2)

Additions to basal, ppm	Weight gain (g)[1] for 26 days	Feather score[1,3]
0	106 ± 5[d2]	4.6 ± .2
54 Ni	123 ± 8[c]	3.6 ± .3
54 Co	133 ± 5[bc]	2.7 ± .4
54 Ni and 54 Co	143 ± 5[b]	2.3 ± .4
60 Zn	175 ± 4[a]	.2 ± .1
60 Zn and 54 Ni	170 ± 6[a]	.5 ± .2
60 Zn and 54 Co	174 ± 6[a]	.6 ± .2

[1,2,3] See footnotes in table 2.

Results

In the first experiment with Ancona chicks (table 2), supplementation of 27 ppm or 54 ppm Ni and Co increased weight gains. All supplemental groups except the 54 ppm Ni group were significantly ($P < .05$) different from the basal group. Feather scores were improved marginally with 27 ppm Ni and 54 ppm Co supplementation. The additions of 27 ppm and 54 ppm Co to the diet appeared to be slightly more effective in increasing weight gain than supplemental Ni, and the 54 ppm Co group was superior to the 27 ppm Co. Supplementation of 60 ppm Zn was adequate for normal feather development and growth, and 30 ppm appeared to be borderline.

In experiment 2, supplementation of 54 ppm Ni or Co to the basal diet improved weight gain significantly ($P < .05$) and reduced feather defects (table 3). The combination of Co and Ni supplementation resulted in almost the same weight gain and feather scores as the Co supplemented group. Addition of 54 ppm Ni or Co to the Zn sufficient diet (+60 ppm Zn) did not promote any more weight gain than the supplemental Zn and resulted in almost perfectly developed feathers as did supplemental Zn alone.

In experiment 3 with New Hampshire X Leghorn cross chicks, supplementation of 54 ppm Ni was in effective in increasing weight gain or reducing feather defects (table 4). However, supplemental 54 ppm Co significantly ($P < .05$) increased weight gain over the basal group and reduced feather score from 3.1 to 1.9. The results of Ni plus Co to the basal diet and of additional

[8] Atomic absorption spectrophotometer, model 403, Perkin Elmer Corp., Norwalk, CT.

TABLE 4. WEIGHT GAIN AND FEATHER SCORE OF NEW HAMPSHIRE LEGHORN CROSS CHICKS FED SUPPLEMENTS OF NI, CO AND ZN (EXPERIMENT 3)

Additions to basal, ppm	Weight gain (g)[1] for 21 days	Feather score[1,3]
0	124 ± 13^{c2}	$3.1 \pm .4$
54 Ni	119 ± 11^{c}	$3.3 \pm .5$
54 Co	160 ± 6^{ab}	$1.9 \pm .5$
54 Ni and 54 Co	158 ± 8^{b}	$2.1 \pm .5$
60 Zn	185 ± 13^{a}	$.3 \pm .2$
60 Zn and 54 Ni	179 ± 5^{ab}	$.5 \pm .3$
60 Zn and 54 Co	181 ± 10^{a}	$1.0 \pm .5$

[1,2,3]See footnotes in table 2.

Ni or Co to the Zn sufficient diet were similar to the effects of supplemental Co and Zn, respectively. A similar observation was made in experiment 2. These observations indicated that there were no additive effects of either Ni to the basal + Co diet, or of Co or Ni to the Zn sufficient diet.

In experiment 4 (table 5), New Hampshire X Leghorn cross chicks fed the 30 ppm Zn supplemental diet grew faster, absorbed a higher percentage of zinc and had better feathering than Ancona chicks fed the same type of diet. The weight gain and ^{65}Zn absorption of New Hampshire X Leghorn cross chicks proved significantly ($P < .001$) different from those of Anconas using the unpaired student t test. Supplementation of 30 ppm Zn was sufficient for feather development of New Hampshire X Leghorn cross chicks but was deficient for Anconas.

TABLE 5. WEIGHT GAINS, FEATHER SCORES AND ^{65}Zn ABSORPTION BY ANCONAS OR NEW HAMPSHIRE X LEGHORN CROSS CHICKS WHEN SUPPLEMENTED WITH 30 PPM Zn (EXPERIMENT 4)

	Additions to basal[4], ppm	Weight gain[1] for 20 days, g	Total mean[1]	Feather scores[1,3]	^{65}Zn absorption[1] (%)	Total mean[1]
	54 Ni	96 ± 4^{c2}		1.7 ± 0.4	21.3 ± 1.5^{cd2}	
	54 Co	97 ± 3^{bc}	99 ± 2	2.0 ± 0.4	23.7 ± 1.9^{bc}	22.3 ± 0.8
Anconas	54 Ni + 54 Co	104 ± 3^{bc}		1.0 ± 0.3	21.9 ± 0.9^{cd}	
	30 Zn	109 ± 4^{b}		1.6 ± 0.3	16.6 ± 1.5^{d}	
	0	145 ± 4^{a}		–	28.0 ± 2.7^{a}	
Hampshire	54 Ni	140 ± 4^{a}		–	32.9 ± 1.9^{a}	
X Leghorn	54 Co	143 ± 4^{a}	143 ± 3**	–	32.1 ± 1.6^{a}	32.7 ± 1.0**
	54 Ni + 54 Co	145 ± 4^{a}		–	33.3 ± 1.8^{a}	

[1,2,3]See footnotes in table 2.

[4]Chicks were fed test diets only during the ^{65}Zn absorption period (2-1/2 days) and a 30 ppm Zn supplemented diet was fed to all groups before and after this period.

**Difference between strains was significant ($P < .001$) by Student's t-test.

In experiment 5 (table 6), the weight gain and feather scores of Ancona and New Hampshire X Leghorn cross chicks showed that the basal diet was severely Zn deficient for Anconas but only marginally Zn deficient for New Hampshire X Leghorn crosses. Supplementation of Co or Ni to the basal diet increased weight gain ($P < .05$) and reduced feather defects with the Ancona strain but was not effective in this experiment with the New Hampshire X Leghorn strain. Supplementation of 54 ppm Co increased ^{65}Zn absorption in New Hampshire X Leghorn chicks and tended to be effective in Ancona chicks, but the difference in Anconas was not significant. Nevertheless, 54 ppm supplemental Ni did not increase ^{65}Zn absorption in either strain of chicks. With supplementation of 60 ppm Zn, the percentage ^{65}Zn absorption was lower. This result could be explained by lower need for zinc and by a greater dilution of ^{65}Zn caused by the Zn supplementation.

TABLE 6. WEIGHT GAINS, FEATHER SCORES AND ^{65}Zn ABSORPTION IN ANCONA AND NEW HAMPSHIRE X LEGHORN CROSS CHICKS FED Ni, Co OR Zr ADDITIONS (EXPRIMENT 5)

	Additions to basal ppm	Weight gain (g) for 24 days	Feather score[1,3]	^{65}Zn absorption %[1]
	0	87 ±3[e2]	4.6 ±.2	18.0 ±.9[c2]
	54 Ni	109± 4[d]	3.9 ±.3	17.4 ±1.0[c]
Ancona	54 Co	111± 4[d]	3.4 ±.3	19.3 ±.3[c]
	60 Zn	154± 4[c]	.5 ±.2	11.7 ±.9[d]
Ancona mean		115± 4	3.1 ±.2	16.8 ±.7
	0	181± 5[b]	.6 ±.2	25.4 ±3.1[b]
Hampshire &	54 Ni	177± 5[b]	.7 ±.2	25.9 ±1.1[b]
Leghorn	54 Co	187± 7[ab]	.5 ±.1	33.1 ±2.5[a]
	60 Zn	199± 7[a]	.2 ±.1	17.7 ±1.2[c]
Hampshire & Leghorn mean		186± 4	.5 ±.1	25.5 ±1.4

[1,2,3]See footnotes in table 2.

New Hampshire X Leghorn cross chicks fed each test diet showed a higher ($P < .05$) weight gain and an increased ($P < .05$) percentage ^{65}Zn absorption compared with Ancona chicks fed the identical diet. Feather scores of New Hampshire X Leghorn cross chicks were superior to those of Ancona chicks, except in the case of the 60 ppm Zn supplemented groups which produced almost perfect feather scores in both strains.

Discussion

Supplementation of Co to the basal diet minimized Zn deficiency signs as indicated by improved weight gain and reduced feather defects with both Ancona chicks and New Hampshire X Leghorn cross chicks. Nickel was effective in some experiments but not consistently. The severe Zn deficiency in the Ancona chicks fed the basal diets may explain why Anconas exhibited more sparing effects of Co or Ni on Zn than did the New Hampshire X Leghorn crosses.

Dahmer (1969) demonstrated that either 50 100 ppm Co or 100 ppm Zn supplementation resulted in improved weight gain and no signs of skin lesions in pigs fed a corn-soybean type diet high in Ca. These results have been confirmed. Furthermore, 50 ppm Co or Ni supplementation was therapeutically effective in treating established Zn deficiency in swine as was 100 ppm Zn supplementation. The effectiveness of supplemental Co or Ni in preventing Zn deficiency was more dramatic in the experiments[3] with pigs than in those with chicks. These results suggest that the sparing effects of Co or Ni on Zn were different in magnitude between species (pigs vs. chicks).

On the other hand, the sparing effects of Co on Zn could not be demonstrated in chicks fed semipurified diets (W.G. Hoekstra, unpublished data), and these effects of Co or Ni have not been shown in rats fed a purified soyprotein based semipurified diet (A.S. Chung, unpublished data). The effects of Co or Ni were also not shown in rats fed a semipurified casein-based diet (Kirchgessner and Pallauf, 1973), or with Ni in rats fed dried egg white as protein source (Spears et al., 1978). These observations suggest that the sparing effects of Co or Ni on Zn nutrition were especially associated with the practical corn-soybean diet rather than with species. Furthermore, phytic acid reduced the bioavailability of Zn in soy protein sources in Japanese quail (Fox et al., 1986). Therefore, phytate help to chelate Zn in soybean and make it partially unavailable. It is possible that Co reduces this bond. The possibility that Co substitutes for Zn in the phytate-Ca interaction is discussed in another paper,[3] and the increased availability of dietary zinc by supplemental Co has been shown in pigs and chicks fed a high Ca corn-soybean type diet[9].

Ancona chicks fed the 30 ppm Zn supplemented diet (total 73 ppm Zn) showed substantial feather defects in experiment 4, while New Hampshire X Leghorn crosses fed the same diet were almost perfect in feather development. Chicks fed a 60 ppm Zn supplemented diet in experiment 5 showed perfect feather development in both strains. In the pig experiments[9] a supplementation of 30 ppm Zn promoted weight gain and no skin lesions. These results were similar to the effects of a 60 ppm supplemental Zn. Ancona chicks may have a requirement which is higher than that of the swine or the New Hampshire X Leghorn cross.

The Zn absorption study showed that Anconas absorbed 22.3% of total Zn intake and New Hampshire crosses absorbed 32.7% in experiment 4 (table 5). A similar superiority of ^{65}Zn absorption was shown in New Hampshire X Leghorn crosses fed diets varying in Co, Ni and Zn content compared with Anconas fed identical diets in experiment 5 (table 6). These results showed that Anconas required more Zn than New Hampshire cross for normal development of feathers and normal growth, and the difference in performance could result from the difference in Zn absorption between the two strains. The high Zn requirement for Ancona chicks was also observed by Englert et al. (1966) and Sunde et al. (1970). Englert et al. (1966) reported that the inbred line of Anconas required higher levels of Zn than some inbred Leghorn lines using the criteria of frayed feathers. Sunde et al. (1970) have shown that the incidence of fraying in this inbred line of Anconas was increased with genetic selection for this abnormality.

The low Zn absorption of Ancona chicks may be related to the low arginine requirement of this strain. Coleman et al. (1971) demonstrated that a high level of arginine aggravated Zn deficiency and that supplemental Zn completely prevented the adverse effects of excess arginine. The arginine requirement for Anconas was lower than that for New Hampshire crosses, about 1.2% vs. 1.6%, respectively (M. L. Sunde, unpublished data). A similar observation was made by Kasarskis (1975) using casein as the protein source. When chicks were fed an arginine deficient diet (total 1.05% arginine), absolute body weight of Anconas was greater than of New Hampshire crosses; at 1.25% arginine, the weight gain of Anconas was not significantly different from that of New Hampshire crosses; and at 1.65% arginine level or more the New Hampshire crosses weighed about 1.3 to 1.4 times more than the Anconas (Kasarskis, 1975). Nesheim and Hutt (1962) showed that genetic differences could affect the arginine requirement of chicks. The corn-soybean diet used in these experiments contained 1.45% arginine by calculation. It seems plausible that this level of arginine could result in the differences in the Zn deficiency signs and Zn requirements between the two strains.

Lease et al. (1960) observed that chicks fed a sesame meal based diet showed leg abnormalities and poor growth, and that 60 ppm supplemental Zn was required to overcome these severe Zn deficiency signs. The sesame meal contains still higher arginine than does soyprotein. Other plant seed proteins are also reported to have a high arginine content (Block and Weiss, 1956). It seems plausible that a high arginine concentration reduces Zn availability from plant seed proteins. This does not imply that phytate and Ca are not important in affecting Zn availability as shown in the review papers (Becker and Hoekstra, 1970; Oberleas, 1973). Kasarskis (1975) demonstrated that arginine was ineffective in alterning the extent of ^{65}Zn absorption in Zn deficient chicks, and similar results were obtained using an isolated duodenal loop *in situ*. His basal diet contained casein as protein source while our basal diet was a practical soybean based diet high in Ca. Therefore, arginine, phytic acid and Ca may have important effects on Zn availability. The difference in Zn absorption between Ancona and New Hampshire X Leghorn cross chicks regardless of dietary Zn level may be explained by the interaction of these factors. Further studies are required to fully document why there is a difference in Zn absorption between Ancona and New Hampshire X Leghorn cross chicks.

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