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# Characterization of cobalt-copper antagonism in the study of copper-stimulated growth in weanling pigs

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ABSTRACT

Two experiments with 96 crossbred pigs each were conducted to study the effectiveness of Co to reduce tissue Cu accumulation in pigs. A 2 x 3 factorial arrangement of treatments, two levels of Cu (15 and 280 mg/kg) and three levels of added Co (0, 150 and 300 mg/kg), was used in both experiments. The initial body weight was 8.2 kg in experiment 1 and 7.2 kg in experiment 2. Tissue samples were taken at d 35 of experiment 1 and d 14 and d 28 of experiment 2 for mineral analysis. Cobalt failed to alleviate Cu deposition in the liver and brain at both dietary Cu levels and increased ($P \leq 0.05$) Cu deposition in the liver of the 150 mg/kg Co group. In contrast, increasing dietary Co linearly decreased ($P \leq 0.05$) Cu deposition in the kidney and Zn deposition in the liver. High dietary Cu increased ($P \leq 0.05$) serum Cu concentration and Cu deposition in the liver, brain and kidney. Copper feeding stimulated ($P \leq 0.05$) growth only during the first week in experiment 1. Dietary supplementation of 150 and 300 mg/kg of Co greatly depressed ($P \leq 0.05$) feed consumption and reduced growth rate ($P \leq 0.05$). In summary, the Cu-Co antagonism is tissue specific and could not be used to prevent liver or brain Cu accumulation. In addition, the Co tolerance level for weanling pigs is lower than 150 mg/kg.

KEY WORDS: copper, cobalt, pigs

## INTRODUCTION

Although dietary supplementation of high levels of Cu is a common practice in the United States swine industry (Ewan, 1986), the mechanism for Cu-stimulated growth is not well understood (Bowland, 1991). Pigs fed high

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levels of Cu in the diet develop excessive Cu deposits in their liver, kidney and other organs (Cromwell et al., 1989; Kornegay et al., 1989), and it is important to understand whether this Cu deposition is necessary for Cu-stimulated growth. An efficient method of removing Cu deposited in tissues is needed to study Cu-stimulated growth.

Sulphide, in the form of ferrous sulphide or sodium sulphide, has been shown to be an effective agent to prevent Cu deposition in the liver of pigs fed high Cu diets (Cromwell et al., 1978; Prince et al., 1979; Ridberiro de Lima et al., 1981). Sulphide, however, could not reduce Cu deposition in the kidney, which suggests that the Cu-sulphide antagonism is tissue specific. It has been reported that injections of inorganic Co greatly enhanced rat urinary Cu excretion (Rosenberg and Kappas, 1989a, b) and reduced the deposition of Cu in the kidney and liver (Rosenberg and Kappas, 1989b). Copper excretion induced by Co was reported to have no accompanying urinary loss of Zn, which is typical of other Cu removing agents (Rosenberg and Kappas, 1989b; Walshe, 1956). Therefore, it seems that inorganic Co could prevent tissue accumulation of Cu by enhancing Cu excretion and thus could be a potential tool for studying the importance of Cu deposition in Cu-stimulated growth.

Very little is known about the effect of Co on pigs. An organic form of Co, vitamin $B_{12}$, is known to be an essential nutrient for pigs (NRC, 1988). Inorganic Co per se is not considered to be essential for pigs (Underwood, 1971). In studying the toxicity of Co in pigs, Huck and Clawson (1976) examined the interaction between dietary Co, Mn, Fe and Zn. They reported that a corn-soyabean diet containing 200 mg/kg of Co did not produce adverse effects. However, antagonistic relationships were found between Co and the three other minerals studied, but their study did not address the potential antagonism between Co and Cu.

The primary objective of this study was to examine the relative long term interactions between Co and Cu in weanling pigs fed high Cu diets.

## MATERIAL AND METHODS

### *Animals and diets*

Two similar feeding trials were conducted, using a total of 192 crossbred weanling pigs (equal numbers of gilts and barrows). Pigs were weaned at a mean age of 35 d and were given a 7-d adjustment period in the nurseries before treatments were started. Pigs were housed in enclosed environmentally regulated rooms in 0.61 m x 0.91 m pens (two pigs/pen) with plastic-coated welded wire flooring. Recommended ventilation rates (Murphy et al., 1990) and tem-

peratures (Harp and Huhnke, 1992) were maintained. The care and treatment of pigs followed published guidelines (Consortium, 1988).

A 20% crude protein maize-soyabean meal-dried whey basal diet (Table 1) was formulated to meet or exceed the National Research Council recommended nutrient requirements (NRC, 1988). Experimental diets were prepared by substituting maize in the basal diet with appropriate levels and copper sulphate or cobalt chloride (Sigma Chemical Co., St. Louis, MO, USA). Pigs were given free access to feed and water during the adjustment and experimental periods.

In experiment 1, 96 weanling pigs (mean body weight of 8.2 kg) were randomly assigned into six treatments from outcome groups based on weight and gender. Littermates were balanced across treatments as much as possible. A 2 x 3 factorial arrangement of treatments was employed using two levels of Cu (15 and 280 mg/kg) and three levels of added Co (0, 150 and 300 mg/kg).

Blood samples were collected via vena cava puncture at the start and at the end of the experiment. During the 35-d experiment, pigs were weighed and feed intake was recorded every week. At the end of the experiment, 24 pigs (four from each treatment) were randomly chosen and killed by electrocution and exsanguination. Whole brain, liver, and both kidneys were collected and stored at $-20^0$C for later analysis of mineral concentrations.

In experiment 2, 96 crossbred pigs (mean BW of 7.2 kg) were randomly assigned to the same dietary treatment arrangement as used in experiment 1. Twenty-four pigs (four pigs per treatment) were killed at d 14 and another 24 pigs were killed at d 28. Body weights were taken and blood samples were taken every week. Brain, liver, and kidney were collected and frozen at $-20^0$C for later mineral analysis.

TABLE 1

Composition (as-is basis) of basal diet for adjustment and experimental periods[a]

| Ingredients | g/kg diet |
|---|---|
| Maize | 55.72 |
| Soyabean meal | 32.22 |
| Dried whey | 10.10 |
| Ground limestone | 0.40 |
| Dicalcium phosphate | 1.11 |
| Trace mineral premix[b] | 0.10 |
| Vitamin premix[c] | 0.25 |
| Salt | 0.10 |

[a] calculated to supply 20 % CP, 0.80 % Ca, and 0.65 % P

[b] supplied per kilogram of diet, mg: Zn, 150; Fe, 175; Mn, 60; Cu, 17.5, and I, 2

[c] supplied per kilogram of diet: retinyl acetate, 1514 $\mu$g; cholecalciferol, 110 $\mu$g; dl-$\alpha$-tocopheryl acetate, 11 mg; riboflavin, 4.4 mg; d-pantothenic acid, 22 mg; niacin, 22 mg; choline 489.5 mg; cyanocobalamin, 22 $\mu$g; menadione, 0.5 mg; d-biotin, 0.44 mg and Se, 0.3 mg

*Mineral analysis*

Serum samples were diluted with distilled water and the Cu concentration was measured using a flame atomic absorption spectrophotometer (Perkin Elmer 5100, Corwalk, CT, USA). Whole organs were homogenized with an Osterizer blender. Samples of the homogenates were wet digested using nitric acid and perchloric acid (AOAC, 1990). Mineral (Cu, Co, Zn and Fe) concentrations were measured by flame atomic absorption spectrophotometry. Certified mineral reference solutions (Fisher Scientific, Fair Lawn, NJ, USA) were used as standards. Dry matter contents of tissue homogenates were also determined (AOAC, 1990). Tissue mineral concentrations are expressed on a dry matter basis.

*Statistical analysis*

Data were analyzed using the GLM procedures of SAS (1988). Pen means were treated as experimental units for analyzing performance data, while individual pigs were used as experimental units for analyzing mineral data. The initial model included Cu level, Co level, Cu x Co interaction, gender and replicates. Gender effects were not significant and thus subsequently removed from the model. Dose response of pig performance to Co was tested in terms of

TABLE 2

Effect of dietary copper and cobalt on serum copper concentration in weanling pigs

| Cu, mg/kg[a] | 15 | 15 | 15 | 280 | 280 | 280 | |
|---|---|---|---|---|---|---|---|
| Co, mg/kg[a] | <2 | 150 | 300 | <2 | 150 | 300 | SEM |
| | | | Experiment 1[b], μg/ml | | | | |
| D 0 | 1.62 | 1.63 | 1.61 | 1.65 | 1.63 | 1.69 | 0.052 |
| D 35[def] | 1.49 | 1.53 | 1.44 | 1.62 | 1.77 | 1.54 | 0.038 |
| | | | Experiment 2[c], μg/ml | | | | |
| D 0 | 1.71 | 1.65 | 1.71 | 1.64 | 1.60 | 1.61 | 0.043 |
| D 7[d] | 1.53 | 1.56 | 1.60 | 1.69 | 1.80 | 1.68 | 0.056 |
| D 14[def] | 1.37 | 1.37 | 1.37 | 1.53 | 1.76 | 1.49 | 0.052 |
| D 21[d] | 1.44 | 1.52 | 1.44 | 1.68 | 1.86 | 1.72 | 0.067 |
| D 28[d] | 1.61 | 1.70 | 1.63 | 1.78 | 2.00 | 1.79 | 0.078 |

[a] upon analysis of feed

[b] each mean represents 16 pigs

[c] each mean represents 16 pigs up to d 14. After d 14, each mean represents eight pigs

[d] Cu effect ($P \leq 0.05$)

[e] Cu level x Co level interaction ($P \leq 0.05$)

[f] quadratic cobalt effect ($P \leq 0.05$)

linear and quadratic effects, using orthogonal polynomial tests. Orthogonal polynomial constans were calculated according to Cady and Fuller (1968).

## RESULTS

### *Serum copper concentration*

Serum Cu concentration was increased by Cu feeding in both experiments (Table 2). In experiment 1, Co influenced the serum Cu concentration in a quadratic manner ($P \leqslant 0.05$), i.e. 150 mg/kg dietary Co elevated Cu concentration whereas 300 mg/kg dietary Co decreased Cu concentration. In experiment 2, the quadratic Co effect was significant ($P \leqslant 0.05$) only at d 14. The magnitude of this quadratic effect was much greater for pigs fed high Cu diets during all periods with a Cu level x Co level interaction ($P \leqslant 0.05$) observed at only d 14 in experiment 2.

### *Tissue mineral concentrations*

Copper feeding increased ($P \leqslant 0.05$) the concentration of Cu in the brain (Table 3) in experiment 1 (d 35), and in experiment 2 at d 28 ($P \leqslant 0.05$), but not at

TABLE 3

Effect of dietary copper and cobalt on brain mineral concentration in weanling pigs

| Cu, mg/kg [a] | 15 | 15 | 15 | 280 | 280 | 280 | |
|---|---|---|---|---|---|---|---|
| Co, mg/kg [a] | <2 | 150 | 300 | <2 | 150 | 300 | SEM |
| | | | Experiment 1 (d 35), μg/g of DM [b] | | | | |
| Cu [c] | 31 | 32 | 32 | 42 | 42 | 44 | 3 |
| Zn | 120 | 121 | 118 | 123 | 118 | 120 | 4 |
| Fe | 58 | 65 | 64 | 66 | 85 | 69 | 9 |
| | | | Experiment 2 (d 14), μg/g of DM [b] | | | | |
| Cu | 21 | 21 | 21 | 21 | 23 | 22 | 1 |
| Zn | 67 | 66 | 65 | 66 | 67 | 66 | 1 |
| Fe | 62 | 67 | 63 | 57 | 62 | 58 | 3 |
| | | | Experiment 2 (d 28), μg/g of DM [b] | | | | |
| Cu [c] | 20 | 21 | 22 | 22 | 25 | 24 | 1 |
| Zn | 66 | 63 | 64 | 63 | 63 | 64 | 1 |
| Fe | 71 | 73 | 75 | 63 | 63 | 55 | 6 |

[a] upon analyses of feed

[b] each mean represents four pigs

[c] Cu effect ($P \leqslant 0.05$)

d 14. Brain Co concentrations were lower than the detection limit of flame absorption spectrophotometry (approximately 2 mg/kg) and were not analyzed. Zinc and Fe concentrations in the brain were not affected by dietary Cu levels. Varying the dietary level of Co did not influence the Cu, Zn, and Fe concentration in the brain.

Concentration of Cu in the liver was increased ($P \leqslant 0.05$) by Cu feeding in both experiments (Table 4). The effect of Co on Cu accumulation in the liver was most evident for pigs fed the high Cu diets. For pigs fed high Cu diets, Co seemed to affect liver Cu deposition quadratically ($P \leqslant 0.05$) in both experiments; the highest liver concentration of Cu was observed for pigs fed 150 mg/kg Co. Cobalt concentration in the liver was linearly increased ($P \leqslant 0.05$) by increasing levels of dietary Co. Copper feeding reduced the accumulation of Co in the liver of pigs fed 300 mg/kg of dietary Co, which can be seen from a Cu effect and a Cu x Co interaction ($P \leqslant 0.05$) at d 35 of experiment 1 and at d 14 of experiment 2. No effect of Cu on Co deposition was observed at d 28 of experiment 2.

TABLE 4

Effect of dietary copper and cobalt on liver mineral concentration in weanling pigs

| Cu, mg/kg [a] | 15 | 15 | 15 | 280 | 280 | 280 | |
|---|---|---|---|---|---|---|---|
| Co, mg/kg [a] | <2 | 150 | 300 | <2 | 150 | 300 | SEM |
| | | | Experiment 1 (d 35), μg/g of DM [b] | | | | |
| Cu [ce] | 39 | 37 | 47 | 745 | 1038 | 884 | 116 |
| Co [ef] | <2 | 9 | 26 | <2 | 10 | 12 | 1 |
| Zn [d] | 231 | 159 | 187 | 252 | 228 | 163 | 20 |
| Fe [c] | 423 | 524 | 573 | 473 | 264 | 228 | 80 |
| | | | Experiment 2 (d 14), μg/g of DM [b] | | | | |
| Cu [ceg] | 85 | 83 | 78 | 150 | 264 | 178 | 25 |
| Co [cdf] | <2 | 11 | 27 | <2 | 10 | 12 | 3 |
| Zn [c] | 171 | 169 | 193 | 129 | 151 | 132 | 23 |
| Fe [ce] | 258 | 448 | 230 | 148 | 200 | 183 | 60 |
| | | | Experiment 2 (d 28), μg/g of DM [b] | | | | |
| Cu [ce] | 24 | 28 | 42 | 398 | 806 | 495 | 111 |
| Co [d] | <2 | 12 | 19 | <2 | 11 | 19 | 4 |
| Zn [d] | 197 | 165 | 130 | 192 | 149 | 140 | 23 |
| Fe [cd] | 363 | 394 | 222 | 255 | 128 | 115 | 43 |

[a] upon analysis of feed
[b] each mean represents four pigs
[c] Cu effect ($P \leqslant 0.05$)
[d] Co linear effect ($P \leqslant 0.05$)
[e] Co quadratic effect ($P \leqslant 0.05$)
[f] Cu x Co interaction ($P \leqslant 0.05$)
[g] Cu x Co interaction ($P \leqslant 0.10$)

Copper feeding reduced ($P \leqslant 0.05$) liver Zn deposition only at d 14 of experiment 2. Generally, Zn concentration in the liver was linearly decreased ($P \leqslant 0.05$) by increasing dietary Co levels except for d 14 of experiment 2. Liver Fe deposition was reduced ($P \leqslant 0.05$) by Cu feeding in both experiments. The effect of Co on liver Fe was not consistent. In experiment 1, the effect of dietary Co on Fe concentration in the liver was not significant (d 35); but at d 14 of experiment 2, Co quadratically influenced Fe concentration with the highest liver Fe concentration occurring in the 150 mg/kg Co groups.

Copper feeding increased ($P \leqslant 0.05$) kidney Cu concentrations at d 35 of experiment 1, but not at d 14 or 28 of experiment 2 (Table 5). In both experiments, kidney Cu concentrations were reduced linearly ($P \leqslant 0.05$) by dietary Co. Cobalt deposition in the kidney was linearly increased by increasing dietary Co level in both experiment ($P \leqslant 0.05$), but was not influenced by Cu supplementation. Concentration of Zn in the kidney was not influenced by dietary Cu or Co levels. High dietary Cu reduced ($P \leqslant 0.05$) kidney Fe concentration only on d 14 of experiment 2. Cobalt feeding increased ($P \leqslant 0.05$) kidney Fe concentration in experiment 2 but not in experiment 1.

TABLE 5

Effect of dietary copper and cobalt on kidney mineral concentration in weanling pigs

| Cu, mg/kg [a] | 15 | 15 | 15 | 280 | 280 | 280 | |
|---|---|---|---|---|---|---|---|
| Co, mg/kg [a] | <2 | 150 | 300 | <2 | 150 | 300 | SEM |
| | | | Experiment 1 (d 35), μg/g of DM [b] | | | | |
| Cu [cd] | 64 | 26 | 25 | 87 | 38 | 34 | 12 |
| Co [d] | <3 | 26 | 55 | <3 | 35 | 64 | 4 |
| Zn | 84 | 64 | 64 | 71 | 71 | 58 | 7 |
| Fe | 165 | 200 | 253 | 210 | 184 | 156 | 2 |
| | | | Experiment 2 (d 14), μg/g of DM [b] | | | | |
| Cu [f] | 40 | 31 | 28 | 34 | 44 | 19 | 25 |
| Co [d] | <5 | 29 | 49 | <5 | 34 | 49 | 3 |
| Zn | 105 | 103 | 118 | 93 | 100 | 96 | 23 |
| Fe [cde] | 119 | 196 | 181 | 96 | 151 | 150 | 60 |
| | | | Experiment 2 (d 28), μg/g of DM [b] | | | | |
| Cu [d] | 36 | 40 | 26 | 76 | 28 | 32 | 10 |
| Co [d] | <5 | 30 | 58 | <5 | 40 | 53 | 6 |
| Zn | 111 | 118 | 107 | 111 | 99 | 105 | 4 |
| Fe [d] | 148 | 177 | 177 | 129 | 111 | 112 | 13 |

[a] upon analysis of feed
[b] each mean represents four pigs
[c] Cu effect ($P \leqslant 0.05$)
[d] Co linear effect ($P \leqslant 0.05$)
[e] Co quadratic effect ($P \leqslant 0.05$)
[f] Co linear effect ($P \leqslant 0.07$)

*Growth performance*

In experiment 1, high levels of dietary Cu stimulated ($P \leqslant 0.05$) growth during d 1 to 7 (Table 6). This occurred through an increase ($P \leqslant 0.05$) in both daily feed intake (ADFI) and gain per feed (GF). No significant Cu x Co interaction in average daily gain (ADG) was found, which indicates that the stimulation of growth was not influenced by Co. During d 1 to 7, Co supplementation linearly decreased ADG at both Cu levels ($P \leqslant 0.05$). ADFI and GF were not influenced by Co treatments. During d 8 to 28, no significant effect of dietary treatments was observed, but during d 29 to 35, both Co and Cu decreased growth rate ($P \leqslant 0.05$). Overall (d 1 to 35), the addition of high levels of dietary Cu did not influence growth performance, whereas dietary Co linearly decreased ($P \leqslant 0.05$) growth rate, which was a result of numerical reductions in ADFI and GF.

In experiment 2, when Co was not supplemented, Cu numerically improved ADG and ADFI during d 1 to 14, but not during d 15 to 28, when Co was not supplemented (compare data column 1 and 4 in Table 6). Dietary Co linearly decreased ($P \leqslant 0.05$) ADG and ADFI regardless of dietary Cu levels. GF was also reduced ($P \leqslant 0.05$) by Co treatment, which indicates that the reduced growth rate was due to a decreased feed intake and reduced feed efficiency. Copper seems to increase the slope of the linear effect of Co. In other words, Cu decreased the growth rate by enhancing the growth inhibitory action of Co (Cu x Co interaction, $P \leqslant 0.08$).

## DISCUSSION

*Copper-cobalt interaction and mineral metabolism*

The interaction between Cu and Co had not been well studied until recently (Rosenberg and Kappas, 1989a, b). The unique ability of Co to increase Cu excretion was observed when Rosenberg and Kappas (1989a) were studying the interaction between Co and a number of metals including Cu. A subcutaneous injection of Co induced a rapid urinary excretion of Cu in rats (within 24 h). A 20% reduction in the Cu concentration of the liver was observed 6 d after Co injection. Our experiments with pigs, however, suggest that Co feeding does not reduce liver Cu accumulation even in long term studies. In fact, in our experiments, Co tended to increase liver Cu deposition, either linearly or quadratically. Cobalt also failed to reduce brain Cu deposition.

Rosenberg and Kappas (1989b) observed that Cu deposition in the kidney was reduced approximately 30% three days after an injection of Co. In our experiments, Co feeding also resulted in a large reduction in kidney Cu

TABLE 6

Effect of dietary copper and cobalt on growth performance in weanling pigs [a]

| Cu, mg/kg [a] | 15 | 15 | 15 | 280 | 280 | 280 | |
|---|---|---|---|---|---|---|---|
| Co, mg/kg [a] | <2 | 150 | 300 | <2 | 150 | 300 | SEM |
| | | | | Experiment 1 [c] | | | |
| D 1-7 | | | | | | | |
| ADG, g [de] | 273 | 221 | 207 | 318 | 325 | 288 | 17 |
| ADFI, g [d] | 417 | 371 | 398 | 468 | 472 | 418 | 19 |
| GF, g/kg [d] | 660 | 594 | 524 | 679 | 686 | 693 | 45 |
| D 8-28 | | | | | | | |
| ADG, g | 445 | 449 | 400 | 508 | 484 | 420 | 25 |
| ADFI, g | 842 | 823 | 756 | 835 | 886 | 791 | 42 |
| GF, g/kg | 529 | 495 | 559 | 534 | 549 | 538 | 20 |
| D 29-35 | | | | | | | |
| ADG, g [def] | 615 | 575 | 510 | 520 | 565 | 358 | 30 |
| ADFI, g | 1251 | 1239 | 1165 | 1170 | 1189 | 1025 | 54 |
| GF, g/kg | 491 | 475 | 441 | 452 | 478 | 354 | 33 |
| D 1-35 | | | | | | | |
| ADG, g [e] | 439 | 394 | 392 | 435 | 466 | 382 | 21 |
| ADFI, g | 826 | 804 | 754 | 818 | 855 | 755 | 32 |
| GF, g/kg | 532 | 498 | 524 | 532 | 547 | 505 | 15 |
| | | | | Experiment 2 [c] | | | |
| D 1-14 b | | | | | | | |
| ADG, g [c] | 259 | 197 | 158 | 304 | 187 | 109 | 32 |
| ADFI, g [c] | 459 | 383 | 325 | 525 | 373 | 306 | 32 |
| GF, g/kg [c] | 579 | 509 | 494 | 561 | 473 | 327 | 46 |
| ED 15-28 c | | | | | | | |
| ADG, g [efg] | 598 | 458 | 397 | 600 | 248 | 172 | 52 |
| ADFI, g [de] | 1037 | 866 | 731 | 1040 | 593 | 489 | 81 |
| GF, g/kg [def] | 579 | 542 | 544 | 586 | 403 | 275 | 53 |

[a] ADG = average daily gain, ADFI = average daily feed intake and GF = gain per feed

[b] upon analyses of feed

[c] each mean represents eight pens with two pigs per pen in experiment 1 and each mean represents eight pens with two pigs per pen to d 14, then there were four pens with one pig per pen and four pens with two pigs per pen in experiment 2

[d] Cu effect ($P \leq 0.05$)

[e] Co linear effect ($P \leq 0.05$)

[f] Co quadratic effect ($P \leq 0.05$)

[g] Cu x Co interaction ($P \leq 0.08$)

concentration (approximately 50% in pigs fed 280 mg/kg dietary Cu), which confirmed a tissue specific Cu x Co antagonism in pigs.

Excessive liver and brain Cu depositions are involved in the pathogenesis of Wilson's disease (Owen, 1981; Wilson, 1982). Inorganic Co was being evaluated as an agent for treating Wilson's desease (Rosenberg and Kappas, 1989b). The failure of Co in pigs to reduce liver or brain Cu deposition raises a doubt about the therapeutic value of inorganic Co, unless humans react differently from pigs. Nevertheless, the unique organ and species specific interaction between Cu and Co is rather interesting.

The major effect of the high level of Cu on liver Co deposition appears to be the prevention of the accumulation of Co when pigs were fed the highest level of dietary Co (300 mg/kg). Because only two levels of dietary Cu were used in our experiments, it is difficult to draw a clear conclusion on the nature of this interaction. Some other minerals are also possibly antagonistic to Co. Iron has been reported to reduce Co absorption in rats (Thomson et al., 1971a,b). A combination of high dietary Fe, Mn, and Zn reduced Co deposition in kidney and liver and alleviated Co toxicity in pigs (Huck and Clawson, 1976). However, the effect of individual minerals on tissue Co in pigs was not studied in that experiment.

Rosenberg and Kappas (1989b) found that Co injection produced a dose dependent elevation of liver Zn in rats. This increase was due to the synthesis of liver metallothionein which binds Zn and increases liver Zn concentration. However, our results show that a high dietary level of Co either had no effect on, or reduced liver Zn levels in pigs. This discrepancy might be due to different routes of administration, i.e., oral administration vs. subcutaneous injections. One important site where mineral antagonism occurs is in intestinal absorption (Wapnir, 1990). It is speculated that dietary Co may interfere with Zn absorption. Even though liver metallothionein synthesis might be induced by Co feeding, Zn storage would still be depleted because of a lower absorption rate or supply of Zn. This is analogous to the Cu x Zn antagonism where Cu feeding reduced liver Zn levels (Hedges and Kornegay, 1973); while Cu injection increased liver Zn storage (Zhou et al., 1994).

The effect of Co on liver Fe storage was studied by Huck and Clawson (1976) who found that dietary supplementation of Co significantly reduced Fe storage in pigs. Our results generally support this conclusion.

*Growth performance*

Statistically significant Cu-stimulated growth was only seen in the first week of experiment 1. In experiment 2, when the two groups receiving no Co supplementation were compared, the high Cu group had numerically improved

performance compared with the low Cu group during the first 14 d. Copper-stimulated growth disappeared in the following weeks. The short term growth stimulation was probably due to an excessive level of dietary Cu. Because diets containing 280 mg/kg of Cu (upon analysis of feed) were used rather than 250 mg/kg commonly used in swine production, it was possible that pigs accumulated too much Cu and marginal Cu toxicity might have developed after the first week. Similarly, Kornegay et al. (1989) found that 400 mg/kg of dietary Cu stimulated the growth of pigs in the first week and the growth stimulation disappeared in the subsequent weeks, presumably because of Cu toxicity.

The growth retardation in pigs by high levels of Co was due to a combination of a decline in feed consumption and feed efficiency, which is consistent with findings of Huck and Clawson (1976). In the study of Huck and Clawson (1976), 200 mg/kg of dietary Co was found to have no detrimental effect on pigs. Based on their report, NRC (1980) recommended 200 mg/kg as the tolerance level of dietary Co for pigs. In contrast, our results showed that 150 mg/kg of Co significantly reduced growth rate, suggesting that tolerance level may be lower than 150 mg/kg for weanling pigs.

Growth performance data from our two experiments were not conclusive on the nature of the interactions between Cu and Co. In experiment 1, the Cu x Co interaction was not significant. In experiment 2, Co linearly depressed ADG of pigs fed both levels of Cu; however, the slope of this linear depression was much greater for pigs fed the high level of Cu. It seems that Cu aggravated the growth depression by Co. This difference may be due to a variable response of pigs to Co. More animals are needed to define clearly the growth response to Cu and Co supplementation.

## CONCLUSION

Contrary to previous reports with rats, Co seemed to be ineffective for preventing brain and liver Cu deposition for pigs. The therapeutic value of Co for treating Wilson's disease in humans needs to be further evaluated. In addition, our data suggest that the maximal Co tolerance level for weanling pigs (approximately 8 kg BW) should be lower than 150 mg/kg diet.

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## STRESZCZENIE

**Antagonizm kobalt – miedź w badaniach nad stymulowanym miedzią wzrostem odsadzonych prosiąt**

Przeprowadzono 2 doświadczenia na 96 mieszańcach, każde dla zbadania wpływu działania Co na obniżenie gromadzenia Cu w tkankach świń. W obydwóch doświadczeniach zastosowano układ czynnikowy 2 x 3, z dwoma poziomami Cu (15 i 280 mg/kg) i trzema poziomami dodanego Co (0, 150 i 300 mg/kg). Początkowa masa ciała prosiąt wynosiła 8,2 kg w doświadczeniu 1 i 7,2 kg w doświadczeniu 2. Próby tkanek do oznaczania składników mineralnych pobierano 35 dnia w doświadczeniu 1 oraz 14 i 28 dnia w doświadczeniu 2. Kobalt nie zmniejszał odkładania Cu w wątrobie i mózgu przy obydwóch poziomach Cu, lecz zwiększał ($P \leqslant 0.05$) odkładanie Cu w wątrobie przy najwyższej dawce Co – 150 mg/kg. Zwiększenie natomiast dawki Co liniowo obniżało ($P \leqslant 0.05$) odkładanie Cu w nerkach i Zn w wątrobie. Wysoka dzienna dawka Cu zwiększała ($P \leqslant 0.05$) koncentrację Cu w osoczu i odkładanie Cu w wątrobie, mózgu i nerkach. Podawanie Cu stymulowało ($P \leqslant 0.05$) wzrost tylko w ciągu pierwszego tygodnia w doświadczeniu 1. Uzupełnienie dawki kobaltem – 150 i 300 mg/kg – znacznie obniżało ($P \leqslant 0.05$) pobieranie paszy i tempo wzrostu ($P \leqslant 0.05$).

Podsumowując, antagonizm Cu-Co jest specyficzny dla poszczególnych rodzajów tkanek i nie może być wykorzystywany do zapobiegania gromadzeniu się Cu w wątrobie i mózgu. W dodatku tolerancja odsadzonych prosiąt na Co jest niższa niż 150 mg/kg skarmianych pasz.