INTERACTIVE EFFECTS OF DIETARY CALCIUM, PHOSPHORUS AND COPPER ON PERFORMANCE AND LIVER STORES OF PIGS¹²

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Summary

Three experiments involving 304 pigs were conducted to determine the related effects of copper (Cu), calcium (Ca) and phosphorus (P) on the performance and liver Cu stores of growing-finishing pigs. Rate and efficiency of gain were improved by the addition of 250 ppm of Cu to the diets. Improvements in rate of gain averaged 6.6% (652 vs 696 g/d) to 60.5 kg body weight and 1.7% (713 vs 725 g/d) to 94.5 kg body weight. Feed:gain ratio was improved by 1.4% to 60.5 kg and 1.6% to 94.5 kg body weight when Cu was added to the diet. Increasing the dietary Ca and P levels from .65% Ca and .55% P to 1.2% Ca and .86 or 1.0% P resulted in increased (P<.01) growth rate to 60 and 95 kg (649 vs 699 g/d and 700 vs 737 g/d, respectively), but feed efficiency was not affected (2.86 vs 2.84 and 3.18 vs 3.17 kg feed/kg gain, respectively.) Feeding the higher Ca and P levels resulted in increased liver Cu levels in pigs fed 250 ppm Cu (189 vs 323 ppm), but Ca and P did not affect liver Cu of pigs fed low Cu diets (29 vs 28 ppm). When dietary Ca and P were varied independently, the high Ca level increased liver Cu, but P had little effect on liver Cu. Increasing the dietary P level partially alleviated the effect of Ca on liver Cu.

(Key Words: Copper, Calcium, Phosphorus, Growth, Liver Copper, Swine.)

Introduction

Addition of 250 ppm of Cu to diets of growing-finishing pigs has been shown by a number of researchers to increase growth rate and decrease the amount of feed required per unit of gain (Braude, 1967, 1975; Wallace, 1967; Cromwell et al., 1978; Prince et al., 1979). The growth response to Cu tends to be less after pigs reach 50 to 60 kg body weight (Miller et al., 1969; Gipp et al., 1973; NCR—42 Committee on Swine Nutrition, 1974), which could be due to the high level of Cu that accumulates in the liver of pigs fed high Cu diets.

Reported values of liver Cu levels of pigs fed 250 ppm Cu are highly variable, indicating that factors other than dietary Cu affect liver Cu levels. Gipp et al. (1973) reported greatly elevated liver Cu (>4,000 ppm) when pigs were fed skim milk diets supplemented with 250 ppm Cu. The skim milk diets contained approximately twice the level of Ca and P of corn-soybean meal diets fed in the same experiments that resulted in more normal liver values. O'Donovan et al. (1966) reported similar results. Carter et al. (1959) and Miller et al. (1959) found that doubling the Ca level of pigs' diets containing 15 ppm Cu resulted in increased liver Cu stores. Guggenheim et al. (1963) reported that feeding additional Ca to mice fed all-meat diets increased liver Cu stores and decreased the severity of Cu-related anemia. Huber and Price (1971) studied Ca and P addition to low Cu diets fed to lactating cows and found that increased Ca alone had little effect on liver Cu, while the addition of P to high Ca diets significantly increased liver Cu stores.

The objectives of these studies were to investigate the effects of dietary Ca and P levels on growth response to Cu and on liver Cu stores of pigs fed 250 ppm of Cu.

Experimental Procedure

Three experiments were conducted utilizing 304 Hampshire-Yorkshire crossbred pigs. All

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pigs were allotted randomly to a randomized complete block design from outcome groups of initial weight within sex with the restriction that littermates be distributed across treatments. Two males and two females were allotted to each pen. Pigs were housed in an open-front shelter with concrete-floored pens and were allowed to consume feed and water ad libitum.

The basal diet consisted of corn and soybean meal fortified with vitamins and minerals (table 1). Copper was added to the diet as copper sulfate (CuSO$_4$·5H$_2$O). Calcium and P were added to the basal diet as calcium carbonate and dicalcium phosphate. The basal diet assayed 13 (Exp. 1) or 17 (Exp. 2) ppm Cu.

Pigs were slaughtered in replicate groups and the central lobe of the liver near the gall bladder was collected from all pigs. The lobe was prepared and analyzed by the method of Kline et al. (1971). Copper was measured by atomic absorption spectrophotometry and is expressed on a dry weight basis. The data were analyzed by variance methods and the pens were considered the experimental units.

Experiment 1 involved a 2 x 2 factorial arrangement of treatments consisting of two levels of added Cu (0 or 250 ppm) and two levels of Ca and P (.65% Ca and .55% P or 1.2% Ca and .86% P). Ninety-six pigs averaging 16.1 kg and 57 d of age were allotted to six replications of four pigs/pen for each treatment. Pigs were slaughtered as each replicate group reached an average body weight of 93 kg. Pigs were on test for an average of 106 d.

In Exp. 2, 128 pigs averaging 13.8 kg body weight and 59 d of age were allotted to eight replications of four treatments. The diet treatments consisted of a factorial arrangement of two levels of added Cu (0 or 250 ppm) and two levels of Ca and P (.65% Ca and .55% P or 1.2% Ca and 1.0% P). Observations from previous experiments had shown that some littermate pigs tended to have liver Cu levels much higher than the average for their respective treatments. To further study these possible genetic effects on liver Cu levels, the randomization of pigs was restricted so that four pairs of pigs from the same litter were distributed across each treatment within a replication. The statistical test used to identify genetic (litter) effects on liver Cu levels was the interaction of treatment x litter set within replication. Replicate groups were slaughtered as pigs reached an average weight of 96 kg. The average time on test was 115 d.

The objective of Exp. 3 was to investigate the interactive effects of dietary Ca and P on performance and liver Cu stores of pigs. Eighty pigs averaging 18 kg body weight and 69 d of age were allotted to five replications of four pigs/pen for each treatment. The treatments consisted of a 2 x 2 factorial arrangement of two levels of Ca (.6 or 1.2%) and two levels of P (.5 or 1.0%). All diets contained 250 ppm added Cu. Pigs were slaughtered in replicate groups at an average weight of 93 kg. The average time on test was 108 d.

Results

Exp. 1. Rate of gain to 66 kg (70 d on test) was greater (P<.05) for pigs fed the high level of Ca and P (table 2). Growth rate was not significantly affected by the level of Cu; however, gains tended to be higher when 250 ppm of Cu was fed (716 vs 703 g/d). A Cu x Ca-P interaction (P<.05) suggested that the level of Ca-P affected the growth response to Cu (4.7% increase in daily gains on low Ca-P diets; 1.0% decrease on high Ca-P diets). The feed:gain ratio to 66 kg was not affected by Cu or Ca-P level. There were no significant differences in final daily gain or feed:gain ratio among the treatment groups although pigs fed the high level of Ca-P did tend to gain faster (730 vs 718 g/d).

Liver Cu levels were increased (P<.01) by the addition of 250 ppm Cu. The high Ca-P level also resulted in increased (P<.05) liver Cu levels in pigs fed diets containing 250 ppm of Cu (401 vs 216 ppm), but neither the main effect of Ca-P nor the Cu x Ca-P interaction was significant (P<.05).

Exp. 2. Daily gain of pigs to 55 kg was increased (P<.01) by the addition of 250 ppm Cu. The high Ca-P level tended to increase liver Cu levels in pigs fed diets containing 250 ppm of Cu (401 vs 216 ppm), but neither the main effect of Ca-P nor the Cu x Ca-P interaction was significant (P<.05).

Liver Cu levels were increased (P<.01) by feeding the high Ca-P diets and by addition of 250 ppm Cu (table 3). High Ca-P treatments also increased (P<.01) rate of gain to 96 kg body weight. Rates of gain to 96 kg were higher (P<.08) for pigs receiving 250 ppm of Cu. Feed:gain ratios to 55 or 96 kg body weight were not affected by Ca-P level or Cu addition.

Liver Cu levels were increased (P<.01) by addition of Cu to the diets. High Ca-P level also resulted in increased (P<.05) liver Cu...
TABLE 1. COMPOSITION OF BASAL DIETS

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Experiment no.</th>
<th>% of diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, ground (IFN 4--02--935)</td>
<td>78.70</td>
<td>79.25</td>
</tr>
<tr>
<td>Soybean meal, dehulled (IFN 5--04--612)</td>
<td>18.65</td>
<td>18.25</td>
</tr>
<tr>
<td>Dicalcium phosphate (IFN 6--01--080)</td>
<td>1.25</td>
<td>1.00</td>
</tr>
<tr>
<td>Calcium carbonate (IFN 6--01--069)</td>
<td>.80</td>
<td>.90</td>
</tr>
<tr>
<td>Salt, iodized (IFN 6--04--051)</td>
<td>.50</td>
<td>.50</td>
</tr>
<tr>
<td>Trace mineral mix&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.05</td>
<td>.05</td>
</tr>
<tr>
<td>Vitamin mix&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.05</td>
<td>.05</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

<sup>a</sup>Contributed per kg of diet: Zn, 100 mg; Fe, 50 mg; Mn, 27.5 mg; Cu, 5.5 mg; I, .75 mg.

<sup>b</sup>Contributed per kg of diet: vitamin A, 2,200 IU; vitamin D<sub>2</sub>, 440 IU; vitamin E, 11 IU; riboflavin, 4.4 mg; pantothenic acid, 11 mg; niacin, 22 mg; vitamin B<sub>12</sub>, 11 μg.

stores, but only in pigs fed the high Cu diets, resulting in a Cu x Ca-P interaction (P<.05).

The interaction of treatment x litter set within replication was not significant, indicating that the variability in liver Cu levels among pigs fed the same diet could not be accounted for by genetic differences.

**Exp. 3.** Growth rate of pigs receiving the high level of Ca was greater than for pigs receiving the lower level (783 vs 756 g/d) but the difference was not significant (P>.05, table 4). A similar trend existed for improvement in efficiency of feed utilization (3.24 vs 3.32). Rates of gain and feed:gain ratios for pigs receiving the two levels of P were similar. There was no evidence of an interaction between Ca and P on rate or efficiency of gain, even though rather large differences in Ca:P ratio existed among the four diets. Pigs fed the highest level of Ca and P did tend to gain more efficiently (3.18) than the other treatment groups.

Liver Cu levels were similar for pigs receiving the two P levels, although P addition to the high Ca diet tended to decrease liver Cu storage.

TABLE 2. EFFECTS OF DIETARY CALCIUM, PHOSPHORUS AND COPPER ON PERFORMANCE AND LIVER COPPER STORES OF PIGS (EXP. 1)<sup>a</sup>

<table>
<thead>
<tr>
<th>Copper, ppm&lt;sup&gt;b&lt;/sup&gt;</th>
<th>0</th>
<th>250</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium, %</td>
<td>.65</td>
<td>1.20</td>
<td>.65</td>
<td>1.20</td>
<td></td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>.55</td>
<td>.86</td>
<td>.55</td>
<td>.86</td>
<td>CV&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Avg daily gain, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>to 66 kg&lt;sup&gt;d&lt;/sup&gt;</td>
<td>682</td>
<td>724</td>
<td>714</td>
<td>717</td>
<td>2.8</td>
</tr>
<tr>
<td>to 93 kg</td>
<td>721</td>
<td>727</td>
<td>715</td>
<td>734</td>
<td>5.8</td>
</tr>
<tr>
<td>Feed/gain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>to 66 kg</td>
<td>2.98</td>
<td>2.93</td>
<td>2.95</td>
<td>2.91</td>
<td>2.4</td>
</tr>
<tr>
<td>to 93 kg</td>
<td>3.16</td>
<td>3.22</td>
<td>3.17</td>
<td>3.12</td>
<td>5.1</td>
</tr>
<tr>
<td>Liver copper, ppm&lt;sup&gt;f&lt;/sup&gt;</td>
<td>32</td>
<td>31</td>
<td>216</td>
<td>401</td>
<td>97.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>Each mean represents six pens of four pigs each, initially averaging 16.1 kg and 57 d of age. Total days on test averaged 106.

<sup>b</sup>Added Cu only. Basal diet analyzed 13 ppm Cu.

<sup>c</sup>Coefficient of variation (100 s/x).

<sup>d</sup>Effect of Ca:P (P<.05).

<sup>e</sup>Cu x Ca-P interaction (P<.05).

<sup>f</sup>Effect of Cu (P<.01).
TABLE 3. EFFECTS OF DIETARY CALCIUM, PHOSPHORUS AND COPPER ON PERFORMANCE AND LIVER COPPER STORES OF PIGS (EXP. 2)*

<table>
<thead>
<tr>
<th>Item</th>
<th>Copper, ppm</th>
<th>Calcium, %</th>
<th>Phosphorus, %</th>
<th>Avg daily gain, g</th>
<th>Feed/gain</th>
<th>Liver copper, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>.65 1.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>.65 1.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avg daily gain, g</td>
<td>576</td>
<td>653</td>
<td>648</td>
<td>705</td>
<td>6.8</td>
<td>49.1</td>
</tr>
<tr>
<td>to 55 kg</td>
<td>669</td>
<td>740</td>
<td>746</td>
<td>746</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>to 96 kg</td>
<td>2.78</td>
<td>2.84</td>
<td>2.72</td>
<td>2.80</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>Feed/gain</td>
<td>3.21</td>
<td>3.20</td>
<td>3.13</td>
<td>3.18</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>Liver copper, ppm</td>
<td>26</td>
<td>26</td>
<td>194</td>
<td>326</td>
<td>49.1</td>
<td></td>
</tr>
</tbody>
</table>

*aEach mean represents eight pens of four pigs each, initially averaging 13.8 kg and 59 d of age. Total days on test averaged 115.

bAdded Cu only. Basal diet analyzed 17 ppm Cu.

cCoefficient of variation (100 s/x).

dEffect of Cu (P<.01).

eEffect of Ca-P (P<.01).

fEffect of Cu (P<.08).

gEffect of Ca-P (P<.05).

hCu X Ca-P interaction (P<.05).

Feeding the high Ca level resulted in a 66% increase in liver Cu levels over the low Ca level (264 vs 159 ppm). However, due to the large variation among liver Cu values, this difference was not significant.

Discussion

In Exp. 1 and 2, feeding 250 ppm Cu resulted in a 6.6% increase (P<.01) in gains during the growing period (to 60.5 kg) and a 1.4% decrease in feed required per unit of gain. For the total test period, rate of gain was increased by 1.7% and feed/gain was decreased by 1.6%. These results are in agreement with the results summarized by Braude (1967, 1975), Wallace (1967), Meyer and Kroger (1973), Cromwell et al. (1978) and Prince et al. (1979) that show Cu to be an effective growth stimulant. The decrease in the magnitude of the growth response during the finishing period is similar to the results of the NCR-42 Committee on Swine Nutrition (1974) and Prince et al. (1979).

The results of these studies indicate that dietary Ca and P levels are not a major contributing factor to the variations in reported growth response to Cu. Although Cu did have a

TABLE 4. EFFECTS OF CALCIUM AND PHOSPHORUS LEVEL ON PERFORMANCE AND LIVER COPPER LEVELS OF PIGS FED 250 PPM COPPER (EXP. 3)*

<table>
<thead>
<tr>
<th>Item</th>
<th>Calcium, %</th>
<th>Phosphorus, %</th>
<th>Avg daily gain, g</th>
<th>Feed/gain</th>
<th>Liver copper, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>.6</td>
<td>.5</td>
<td>754</td>
<td>3.30</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>.5</td>
<td>758</td>
<td>3.33</td>
<td>169</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>.5</td>
<td>781</td>
<td>3.30</td>
<td>300</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>.5</td>
<td>785</td>
<td>3.18</td>
<td>228</td>
</tr>
</tbody>
</table>

*aEach mean represents five pens of four pigs each, initially averaging 18 kg and 69 d of age. Total time on test averaged 108 d.

bCoefficient of variation (100 s/x).
greater beneficial effect when added to low Ca-P diets than to higher Ca-P diets in Exp. 1, the Cu × Ca-P interaction was not significant (P>0.05) when data from Exp. 1 and 2 were pooled.

The increased daily gains from higher Ca-P levels was not expected as the lower levels were estimated to be adequate to optimize performance. However, when the results of Exp. 1 and 2 were pooled, rates of gain were greater (P<0.01) for the pigs fed the high Ca-P level at both intermediate and final weights. Results from Exp. 3 indicate that the increased gains were more likely due to an increase in Ca rather than P level. These data are in contrast to those of previous experiments (Cromwell et al., 1970, 1972; Parker, 1975) that showed no improvement in performance when Ca or P level was increased above the lower levels tested in these experiments.

The addition of 250 ppm Cu to the diet markedly increased (P<0.01) the average amount of Cu stored in the liver from 28 to 312 ppm. These levels are similar to those reported by Kline et al. (1971), NCR-42 Committee on Swine Nutrition (1974) and Prince et al. (1979). Feeding high levels of Ca-P increased the storage of Cu in the liver in both Exp. 1 and 2, but the effect was significant only in Exp. 2.

The variances for liver Cu levels among treatments were found to differ (P<0.05) due to the greater variability of liver Cu level in pigs fed 250 ppm added Cu as compared with those of pigs on diets without added Cu. Thus, to have more valid estimates of the effects of Ca and P on liver Cu levels, the data from pigs fed diets containing 250 ppm added Cu were analyzed separately. Though the Ca and P levels varied some among experiments, the data for all three experiments were pooled for this analysis and considered as high vs low Ca and P. On this basis, the higher Ca and P increased liver Cu stores in pigs fed diets containing 250 ppm Cu (189 vs 323 ppm).

The results of Exp. 3 suggest that Ca was largely responsible for the elevated liver Copper levels in pigs fed the high Ca-P diets. The data also suggest that increasing the P level partially alleviates the effect of high dietary Ca on liver Cu stores.

The inhibitory effect of high dietary Ca on Zinc (Zn) absorption from the gastrointestinal tract is well known (Underwood, 1971). Zinc also has been shown to interfere with Cu uptake by liver tissue (Ritchie et al., 1963), due to competition of Zn and Cu for common binding sites on the hepatic storage protein, metallothionein (Evans, 1973). Although our diets were supplemented with sufficient Zn (100 ppm) to meet or exceed NRC (1979) standards, the higher Ca level may have reduced Zn absorption sufficiently to result in lower Zn levels in the liver, thereby allowing an increased uptake of Cu by liver tissue.

The magnitude of the differences in liver Cu levels reported herein are not sufficiently large to suggest that high dietary Ca or P levels cause such high levels (4,657 ppm) as those reported by Gipp et al. (1973). Although dietary Ca or P may account for a portion of the variation, other factors or combinations of factors must be responsible for the major portion of liver Cu variation seen in these and other trials.

**Literature Cited**


