# Effect of Iron and Copper Intake on Iron, Copper, and Myoglobin Levels in Selected Pig Tissues

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During two trials, pigs in three lots were fed: Lot I, control; Lot II, control plus Fe; Lot III, control plus Fe and Cu (Cu:Fe proportions 6:1, 44:1, and 10:1, respectively). Frozen tissue samples were subjected to a wet ash digestion and Fe, Cu, and myoglobin levels were determined spectrophotometrically. Iron levels of muscles Lots I and III were not significantly different, but were significantly higher than in Lot II. Psoas major and biceps femoris muscles contained significantly more Fe than other muscles studied. There were no significant differences in Cu levels among the muscles. Myoglobin levels were significantly different among muscles, but not among lots; muscles with highest myoglobin levels did not necessarily have highest Fe levels. There were no differences in Fe or Cu levels in liver lobes. In Trial 2, the Fe mean of Lot I was significantly lower than of Lots II and III, whose differences were nonsignificant. Cu levels in livers in Lots I and III were not significantly different but were significantly higher than in Lot II. There were no differences in Fe levels of spleens among lots. The spleen Cu level in Lot I was lower than in Lots II and III, which were similar.

Since iron and copper deficiencies are recognized in animal nutrition, feeds supplemented with minerals were suggested to improve the conditions (*2*, *7*, *10*, *11*, *14*).

This study investigated iron, copper, and myoglobin content of selected muscles, and iron and copper content of livers and spleens from pigs fed the experimental rations. Studies on the distribution and accumulation of ingested minerals in parts of animal bodies fed a relatively high mineral diet may help to elucidate unknown factors that contribute to better feed utilization and growth and consequently improved meat quality and quantity.

## *Materials and Methods*

Tissues of the muscles, livers, and spleens used for chemical analyses were obtained from pigs in two feeding trials. Muscles were examined for iron, copper, and myoglobin, and livers and spleens for copper and iron. Copper was determined in the second trial only.

**History of Animals.** Piglets 2 or 3 days old were injected intramuscularly with 50 mg. of iron-dextran to prevent baby pig iron deficiency. Subsequently, the animals were fed with a nonpurified ration until they were placed on feeding experiments, but no additional iron and copper were supplied by any means. In both trials, the pigs were approximately 8 weeks old and weighed 18 kg. (average) when assigned to lots. Hemoglobin levels, checked before the pigs were placed on trial, were in the normal range. All pigs were wormed before the trial and no differences in parasite load at slaughter were discernible.

In Trial 1, four Duroc gilts were randomly assigned to each of three lots; in Trial 2, crossbred Yorkshire, Duroc, and Poland China pigs were placed in three lots: nine in Lots I and II, and eight in Lot III, including barrows randomly selected in each lot.

Identical pelleted rations were fed *ad libitum* in both trials (Table I). Zinc was supplied at 50 p.p.m. in the form of zinc oxide to prevent parakeratosis. The control ration (Lot I, sorghum grain and soybean oil meal) contained 16% protein and provided 88 p.p.m. iron and 15 p.p.m. copper. Pigs in Lots II and III were fed the control ration supplemented with ferrous sulfate to provide 661 p.p.m. of iron. Cupric sulfate was added to the ration of Lot III to supply 66 p.p.m. of copper. The ratios of iron to copper were: Lot I, 6:1; Lot II, 44:1; and Lot III, 10:1.

**Table I. Composition of Rations**

| Lot No. | Ingredients | % |
|---|---|---|
| I | Control ration[a] | |
| | Sorghum grain | 79.0 |
| | Soybean oil meal | 9.5 |
| | Alfalfa meal | 5.0 |
| | Meat scraps | 5.0 |
| | Aurofac[b] | 0.5 |
| | Iodized salt | 0.5 |
| | Zinc oxide[c] | 0.0063 |
| II | Control ration[a] + ferrous sulfate[c,d] | |
| III | Control ration[a] + ferrous sulfate[c,d] + cupric sulfate[c,e] | |

[a] Contained 88 mg. Fe and 15 mg. Cu/kg. of ration.
[b] Commercial aureomycin and vitamin $B_{12}$.
[c] Courtesy of Calcium Carbonate Co., Quincy, Ill
[d] Contained 573 mg. Fe/kg. of ration.
[e] Contained 51 mg. Cu/kg. of ration.

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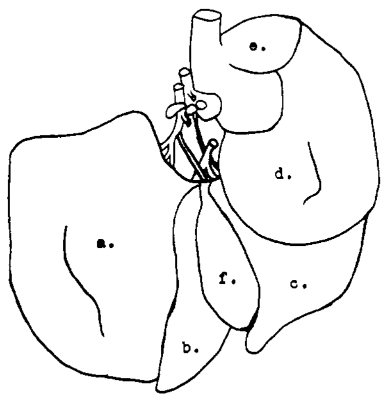


Figure 1. Diagram of pig liver

*a.* Left lateral, lobe 1
*b, c.* Left and right central, lobe 2
*d.* Right lateral, lobe 3
*e.* Caudate lobe, not tested
*f.* Gall bladder

The pigs were housed on concrete floors, penned with galvanized wire, and water was available at all times in wooden troughs. Iron in the drinking water was approximately 0.03 p.p.m. The level of copper in the water was not known; however, no copper tubing was used to supply water. Hogs were individually removed from trial as they reached 95 kg., live weight, and were slaughtered in the university meat laboratory.

**Mineral Analyses.** Laboratory samples of muscles to be studied were dissected from the right side of each chilled carcass, and freed from adhering tissues, including the epimysium. Samples from livers and spleens were taken as the organs were removed. Stainless steel knives were used in the sampling, and all operations were performed on wooden-top tables. Livers were laid gall bladder side up with the distal ends of the lobes toward the sampler (Figure 1). A longitudinal core approximately 1 cm. square was taken from each muscle and from each of the three lobes of the livers for analyses. When the spleens were large enough, cores of the same size were taken from each, avoiding the midline and adhering tissues. The experi-

**Table II. Mean Values of Iron and Myoglobin**

| | Muscles and Trial Numbers | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Psoas major | | Biceps femoris | | Longissimus dorsi | | Rectus femoris | | Semimembranosus | | Lot Means | |
| Lot No. and Diet | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| **Iron, Micrograms per Gram of Muscle** | | | | | | | | | | | | |
| Lot I control | 12.0 | 14.2 | 15.5 | 13.1 | 10.3 | 8.4 | 8.3 | ... | 7.3 | ... | 10.7[a] | 12.9 |
| Lot II control + iron | 9.4 | 14.6 | 9.4 | 10.1 | 5.2 | 7.6 | 4.9 | ... | 6.3 | ... | 7.0[a] | 10.8 |
| Lot III control + iron + copper | 12.4 | 13.6 | 12.2 | 12.0 | 9.6 | 13.1 | 9.0 | ... | 5.3 | ... | 9.7[a] | 12.9 |
| Muscle means | 11.3[b] | 14.1[c] | 12.4[d] | 11.7[c] | 8.4[d] | 9.7[c] | 7.4[b,d] | ... | 6.3[b,d] | ... | ... | ... |
| **Myoglobin, Milligrams per Gram of Muscle** | | | | | | | | | | | | |
| Lot I control | 0.63 | 0.62 | 0.20 | 0.31 | 0.13 | 0.16 | 0.43 | 0.54 | 0.18 | 0.23 | 0.31 | 0.33 |
| Lot II control + iron | 0.77 | 0.62 | 0.26 | 0.33 | 0.10 | 0.16 | 0.64 | 0.69 | 0.15 | 0.20 | 0.38 | 0.40 |
| Lot III control + iron + copper | 0.85 | 0.81 | 0.23 | 0.32 | 0.07 | 0.16 | 0.58 | 0.51 | 0.16 | 0.22 | 0.38 | 0.40 |
| Muscle means | 0.75[e] | 0.68[f] | 0.23[e] | 0.32[f] | 0.10[e] | 0.16[f] | 0.55[e] | 0.58[f] | 0.16[e] | 0.22[f] | ... | ... |

Least significant differences (lsd) for muscles at 5% level, by Trial

| Trial 1 | | Trial 2 | |
|---|---|---|---|
| PM *vs.* BF, lsd = 0.27 | BF *vs.* RF, lsd = 0.21 | PM *vs.* BF, lsd = 0.12 | BF *vs.* RF, lsd = 0.17 |
| PM *vs.* LD, lsd = 0.26 | BF *vs.* SM, lsd = 0.07 | PM *vs.* LD, lsd = 0.13 | BF *vs.* SM, lsd = 0.05 |
| PM *vs.* RF, lsd = 0.33 | LD *vs.* RF, lsd = 0.21 | PM *vs.* RF, lsd = 0.20 | LD *vs.* RF, lsd = 0.17 |
| PM *vs.* SM, lsd = 0.27 | LD *vs.* SM, lsd = 0.06 | PM *vs.* SM, lsd = 0.12 | LD *vs.* SM, lsd = 0.06 |
| BF *vs.* LD, lsd = 0.06 | RF *vs.* SM, lsd = 0.21 | BF *vs.* LD, lsd = 0.06 | RF *vs.* SM, lsd = 0.17 |

[a] To compare means, use a difference of 2.5 as significant at 5% level.
[b] To compare means, use a difference of 3.8 as significant at 5% level.
[c] To compare means, use a difference of 3.8 as significant at 5% level.
[d] To compare means, use a difference of 3.2 as significant at 5% level.
[e] To compare means, use Trial 1 lsd's.
[f] To compare means, use Trial 2 lsd's.

mental samples were immediately wrapped in individual aluminum foils, labeled appropriately, frozen on a plate freezer at −23° C. and stored at temperatures between −18° and −12° C. in a freezer.

A wet-ash digesting procedure using sulfuric and perchloric acids was applied to analytical tissue samples weighing between 0.05 and 0.20 gram. Four reagent blanks and four standards were carried through with each assay. Iron values were determined spectrophotometrically using a modification of methods by Saywell and Cunningham (*20*) and Smith, McCurdy, and Diehl (*21*). The color reagent used was 4,7-diphenyl-1,1,10-phenanthroline in 95% ethyl alcohol. To determine copper values, a modified method of Rice (*18*) was used. The color reagent was a saturated solution of oxalyldihydrazide in concentrated ammonium hydroxide.

The values obtained were recorded in micrograms of metal per gram of sample. All data were subjected to analysis of variance, and when appropriate, least significant differences, $P = 0.05$, were calculated.

An adaptation of the method by Poel (*17*) for myoglobin concentration in striated muscle was applied, using potassium ferricyanide and carbon monoxide. Analysis of variance was done on the mean myoglobin values for each muscle, and least significant differences were determined between muscles.

## *Results and Discussion*

Rations with supplemental iron and copper accelerated weight gains, raised meat grades, improved tenderness and induced dark red color in veal (*3*, *9*, *15*). Copper was shown to increase feed consumption and utilization, growth rate, and dressing percentage significantly, but to decrease carcass length (*1*, *4*). Supplementing feed with iron, manganese, copper, cobalt, and magnesium decidedly increased size and improved appearance of beef muscles (*12*, *13*). However, overdoses of iron and copper in pig rations caused metabolic disturbances, weight decreases, and toxicity (*16*, *19*).

The color of meat is thought to be related to the amount of myoglobin in muscle tissues (*6*). Veal muscles are light red and beef, dark red; however, there is little difference in iron per gram between beef and veal muscles (*22*). A particular color is associated with individual pork muscles; the psoas major, gluteus accessorius, and gluteus profundus are dark red, but most other muscles are grayish pink.

Mean values of iron, copper, and myoglobin in muscles, and iron and copper levels in livers and spleens indicated no differences between sexes or among breeds.

**Muscle Iron Levels.** Iron levels of the five pork muscles studied are presented in Table II. In Trial 1, the level in biceps femoris (BF) was significantly higher than in the longissimus dorsi (LD), rectus femoris (RF), or semimembranosus (SM); the iron level in psoas major (PM) was significantly higher than in either RF or SM. In Trial 2, the level of iron in PM was significantly higher than in LD, but the level in BF was not significantly different from that in LD. In both trials the concentrations of iron in PM and BF were higher than in other three types of muscles studied.

The mean iron level for all five muscles for pigs fed the control ration in Trial 1 did not vary significantly from that of pigs fed the control ration supplemented with iron and copper. Moreover, the iron levels in both lots were significantly higher than in pigs fed the control ration plus iron. In Trial 2, iron levels were determined in three muscles only. The mean iron level was similar for Lots I and III. Although the mean level in muscles of Lot II was low, it was not significantly different from Lots I and III.

**Muscle Copper Levels.** According to Table III, differences in copper levels were nonsignificant among types of muscles. The muscle mean of PM was highest in copper level, followed by lower levels in BF and LD. Also, copper levels in treatment means were nonsignificant; however, Lot II was higher than Lots I and III.

**Muscle Myoglobin Levels.** Myoglobin levels of five pork muscles from Trials 1 and 2 are reported in Table II. The BF myoglobin level was significantly lower than those in PM or RF. The PM and RF myoglobin levels did not differ significantly from each other. The level of myoglobin in LD was lowest of the five muscles and significantly different from SM in both trials.

**Table III. Mean Values of Copper in Muscles, Livers, and Spleens (Micrograms per Gram, Trial 2 Only)**

| Lot No. and Diet | Muscles | | | | Livers | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Psoas major | Biceps femoris | Longissimus dorsi | Lot means | Lobes 1 | Lobes 2 | Lobes 3 | Lot means | Spleens |
| Lot I control | 3.3 | 2.4 | 2.7 | 2.8 | 8.0 | 8.6 | 8.4 | 8.4[a] | 2.3[b] |
| Lot II control + iron | 3.1 | 4.0 | 3.5 | 3.5 | 7.3 | 7.9 | 8.0 | 7.7[a] | 2.9[b] |
| Lot III control + iron + copper | 3.4 | 3.0 | 2.7 | 3.0 | 8.3 | 8.9 | 8.7 | 8.6[a] | 2.9[b] |
| Muscle and lobe means | 3.3 | 3.1 | 3.0 | | 7.9 | 8.5 | 8.4 | | |

[a] To compare means, use a difference of 0.5 as significant at 5% level.
[b] To compare means, use a difference of 0.4 as significant at 5% level.

The variation of mean myoglobin levels in Lots II and III for all five muscles of pigs was nonsignificant in both trials. The myoglobin level of Lot I was lowest of the three lots.

**Liver and Spleen Iron Levels.** The data presented in Table IV revealed no significant differences in iron levels of animal livers from the three lots of Trial 1. However, iron level in livers from pigs fed the control ration in Lot I was lowest of the three lots. In Trial 2, no significant differences were observed between Lots II and III, but the level of iron in the liver of pigs of Lot I was significantly lower than in Lots II or III. No significant differences in iron levels among the three lobes or liver or in the spleen were observed in either trial.

**Liver and Spleen Copper Levels.** Copper levels for livers and spleens are presented in Table III; differences among lots for copper levels were significant. The liver mean of Lot II was lower than Lots I or III. Copper levels among the three liver lobes were not significantly different.

Copper levels in spleens were lower than in livers. Spleens of Lot I contained significantly less copper than those of Lots II or III.

**Iron and Myoglobin Interrelationships.** An increase of iron in the ration in Lot II decreased the iron level in muscles of pigs (Table II). Myoglobin increased when iron was added to the control ratio (Table II). Iron levels in muscles from animals in Lots I and III and myoglobin levels in Lots II and III did not differ significantly.

Among individual types of muscles, iron and myoglobin levels decreased in SM with increased iron in the ration. In Trial 1 iron decreased and myoglobin increased in PM muscle when iron was added to the ration. In Trial 2, neither iron nor myoglobin increased.

The dark red color of the PM could be attributed to high myoglobin level from the high iron level in the muscle. Neither RF nor BF muscles are dark red; nevertheless, the RF showed high myoglobin level associated with a low iron, and BF, an intermediate amount of myoblobin with high iron level. The LD, a definite grayish pink muscle, had the lowest myoglobin level of all muscles studied and intermediate iron level.

The data presented here indicate that color of individual pork muscles is not directly related to their iron or myoglobin levels except when both components are high, as in PM. Evidently, iron and myoglobin accumulate independently of each other in individual types of muscles.

**Iron and Copper Interrelationships.** Iron decreased in muscles of pigs fed the control ration supplemented with iron, but copper increased (Figure 2). When iron and copper were added to the control ration, iron and copper levels in the muscles were similar to those in the control treatment. Iron supplements increased iron accumulation in the liver, but iron and copper supplementation increased both. Iron supplements did not affect iron level of the spleen, but increased copper; supplements of iron and copper increased levels of both.

The data obtained indicate that the iron-to-copper ratio affected either absorption of the metals from ingested food or their translocation from metabolic sites in the animal body. High dietary iron could increase copper requirement and copper concentration at active sites of iron metabolism, other than the liver. The 44:1 supplement decreased iron in the muscles but increased it in the liver, which confirms Gubler's observations (*8*).

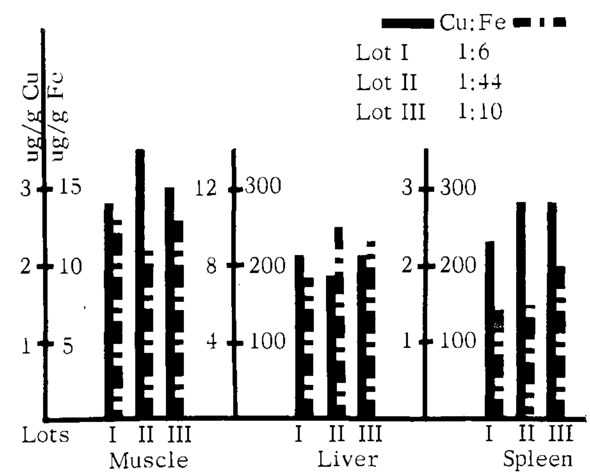


Figure 2. Retention of iron and copper by pork tissues

**Table IV. Mean Values of Iron in Livers and Spleens**
**(Micrograms per Gram)**

| Lot No. and Diet | Liver Lobes and Trial Numbers: Lobe 1, 1 | Lobe 1, 2 | Lobe 2, 1 | Lobe 2, 2 | Lobe 3, 1 | Lobe 3, 2 | Livers, Lot Means, 1 | Livers, Lot Means, 2 | Spleens, Trials, 1 | Spleens, Trials, 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| Lot I control | 94.1 | 163.7 | 97.0 | 198.7 | 144.6 | 168.4 | 111.6 | 177.5[a] | 143.0 | 141.9 |
| Lot II control + iron | 184.8 | 253.6 | 202.1 | 246.1 | 171.1 | 247.3 | 186.0 | 256.1[a] | 342.6 | 146.2 |
| Lot III control + iron + copper | 166.2 | 277.4 | 143.2 | 201.6 | 165.1 | 227.4 | 158.2 | 233.3[a] | 260.0 | 198.2 |
| Lobe means | 148.4 | 198.2 | 147.4 | 215.5 | 160.3 | 214.4 | | | | |

[a] To compare Trial 2 liver means, use a difference of 45.2 as significant at 5% level.

The high iron ration was associated with increased copper in muscles and spleen and decreased copper in the liver.

At a lower iron to copper ratio (10:1) in the feed, the metals accumulated in muscles as with the control ration, which had a narrow ratio (6:1) of the metals. In the liver, the 10:1 ratio showed a reverse narrow relationship of accumulated metals over the control. However, the difference in accumulation was not so great as when the ratio of metals was 44:1.

Ritchie *et al.* (*19*) and Cassidy and Eva (*5*) reported inverse relationships of iron and copper accumulation in pig liver when animals were fed high dietary copper. The authors' data confirm their observations that an acute relationship exists between the two metals. The information will help animal nutritionists prepare improved rations for meat-producing animals.

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