Exploratory Study: Excessive Iron Supplementation Reduces Zinc Content in Pork without Affecting Iron and Copper

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Simple Summary: Currently, all pigs raised on intensive farms develop iron-deficiency anemia if they do not receive supplemental iron at birth. Weaning diets commonly contain high concentrations of iron, and the effect on the copper and zinc contents in pork is unknown. In this exploratory work, we determined the effect of excessive oral iron supplementation on the contents of these microminerals in pork. Surprisingly, we found that high iron doses of 3000 ppm reduced the zinc content of pork by 32–55%.

Abstract: The aim of this work was to determine in an exploratory manner the effect of excessive iron supplementation on iron, zinc, and copper contents in pork and pork offal. Pigs averaging 50 days in age and 15 ± 1.3 kg body weight were allocated to a control group (500 ppm dietary Fe) and a supplemental group (3000 ppm dietary Fe). After an iron supplementation period of 60 days, blood samples were analyzed to determine iron biomarkers, serum copper, and zinc contents. Animals were slaughtered to assess total iron, non-heme iron, heme iron, zinc, and copper contents in samples of nine meat cuts and some offal. Iron supplementation improved the iron status in pigs with increased hemoglobin and hematocrit, but did not affect serum levels of iron, zinc, and copper. Iron supplementation did not affect the heme and non-heme iron contents of the different meat cuts. Zinc contents decreased by 32–55% in meat cuts, where iron content increased in the liver, spleen, kidneys, and pancreas. No differences of zinc and copper were observed in offal samples. High concentrations of iron supplementation reduce zinc content in pork.

Keywords: copper; iron; supplementation; pigs; pork; offal; zinc

1. Introduction

Iron, zinc, and copper are trace minerals that play a key role in several biological processes. These microminerals are found mainly in foods of animal origin (e.g., meats and shellfish). Meat and shellfish are rarely consumed in developing countries, resulting in major nutritional deficiencies [1–3]. Iron deficiency is the most prevalent nutritional disorder, affecting up to 30% of the world’s population [4]. Iron deficiency can be treated with increased meat consumption [5]. Meat products provide several essential nutrients including proteins, lipids, minerals, and micronutrients, and 50–60% of the total iron present in pork is heme iron, which is a highly bioavailable form of iron [6]. Zinc found in meat is absorbed better than zinc present in vegetables [7].

Mineral supplementation in pigs has been widely researched, especially iron, because of the high prevalence of iron-deficiency anemia that develops between the first and
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second week of age in pigs raised under intensive conditions. The iron deficiency becomes more acute three weeks after weaning, where the prevalence of anemia (6–32%) and iron deficiency (29–74%) increases [8]. This condition results in low live weights and daily weight gain, impaired functioning of the immune system [9], and greater susceptibility to diseases [10]. Different iron supplementation strategies have been investigated in suckling and weaned piglets [11–13]. However, an excess of iron can be harmful to pig health, as it facilitates free radical formation [14], increases the incidence of diarrhea [15], alters iron homeostasis, increases hepcidin to critical levels [16], and causes cognitive impairments [17].

The effect that excessive iron supplementation can have on other microminerals in meat has been poorly studied. Doses of iron greater than 1000 ppm in a pig’s diet are considered high and increase the destruction of tocopherols [18]. However, a dose of 3000 ppm does not affect the performance of pigs. At a dose of 4000 ppm, the growth rate and inorganic phosphorus in serum are reduced. With 5000 ppm, the ash content of the femur is also diminished [19,20]. A dose of 3000 ppm is the maximum tolerable concentration that does not diminish animal performance [21]. For these reasons, we used 3000 ppm of iron in this study. We hypothesized that excessive oral supplementation with 3000 ppm iron could influence the deposit of other minerals in pork, such as zinc and copper, which are absorbed through similar pathways in the intestine. The aim of this work was to determine the effect of excessive iron supplementation on iron, zinc, and copper contents in pork and pork offal.

2. Materials and Methods

2.1. Animals

Eight commercial hybrid male pigs (TEMPO × TOPIG 20) were used, averaging 50 days in age and 15 ± 1.3 kg body weight. All protocols were approved by the Bioethics Committee from the Institute of Nutrition and Food Technology (INTA) at the University of Chile (Certificate of Bioethics 501-11, project FONDECYT # 1095038). For the calculation of the sample size, an error of α = 0.05, a power of 80%, a difference between the control group and the supplemental group of 0.8 mg iron/100 g of the loin cut, and a standard deviation of 0.3 were considered. The calculated N was 4 animals in each group.

2.2. Experimental Design

Animals were randomly assigned to one of two groups: (1) the control group (Fe500, n = 4), where pigs were fed a standard diet based on NRC recommendations [22] (Table 1) that contained 500 ppm of iron; or (2) the supplemented group (Fe3000, n = 4), where pigs were fed the same diet described above supplemented with ferrous sulfate (Veterquimica S.A, Santiago, Chile) with up to a total iron content of 3000 ppm. Food intake was controlled: in the first 20 days, each animal received 700 g/day; in the following 20 days, they received 1 kg/day; and in the last 20 days, they received 1.5 kg/day. All meals were divided into two rations per day.

The proximate composition of the diets was analyzed according to the methodology proposed by the Association of Official Analytical Chemists [23] for moisture (method 945.15), crude protein (Kjeldahl method 945.18, N × 6.25), ether extract (method 945.16), crude fiber (method 962.09), and ash (method 920.153). Meanwhile, iron, copper, and zinc contents were determined following the directives for the acid digestion method proposed by AOAC (1996). Mineral concentrations were measured at specific wavelengths for each element (iron: 248.3, copper: 324.7, and zinc: 213.9) using an atomic absorption spectrophotometer with graphite furnace (SIMAA 6100, PerkinElmer, Waltham, MA, USA).

Each pig was weighed individually on days 0 and 60. The mean weight gain at the end of experimental period of 60 days was calculated.
Table 1. Diet composition (as-fed basis).

| Ingredient      | g/kg |
|-----------------|------|
| Corn            | 610.0|
| Soybean         | 200.0|
| Olein           | 15.0 |
| Sorghum         | 55.0 |
| Bigolac®        | 20.0 |
| Fish meal       | 32.0 |
| Wheat bran      | 50.0 |
| Oyster shell    | 5.0  |
| Phosbic®        | 3.0  |
| Premix a        | 3.0  |
| Salkil®         | 2.0  |
| Lysine          | 3.0  |
| Threonine       | 2.0  |
| Natuphos®       | 0.5  |
| Salt            | 0.5  |

Composition (%)

| Component       | Amount |
|-----------------|--------|
| Crude protein   | 18.7   |
| Ether extract   | 6.9    |
| Crude fiber     | 4.2    |
| Ash             | 5.8    |
| Free-nitrogen extract | 54.5 |

*a Vitamins and minerals (per kg of premix): Vitamin A (9900 UI), Vitamin D (1650 UI), Vitamin E (77 UI), Vitamin K (4.4 mg), choline (330 mg), niacin (44 mg), riboflavin (9.9 mg), B12 (44 mcg), folic acid (770 mcg), biotin (154 mcg), thiamin (3.3 mg), pyridoxine (4.4 mg), Ca (33 mg), Zn (130 g), Mn (45 g), Cu (15 g), I (0.55 g), and Se (0.30 g). The iron content in the control and supplemental groups was 500 and 3000 ppm, respectively.

2.3. Hematological Parameters and Micromineral Status

To assess mineral status, 30 mL blood samples were drawn from piglets by jugular venepuncture at days 0 and 60. Total blood was used to determine the following biomarkers of iron nutrition status: hemoglobin, hematocrit, and mean corpuscular volume (Cell-Dyn 3200; Abbott Laboratories, Abbott Park, IL, USA). Serum was separated by centrifugation of whole blood at 1200 × g for 3 min, and then immediately stored at −20 °C. Total iron binding capacity (TIBC) was determined in the serum by a colorimetric method [24]. Serum iron, zinc, and copper concentrations were determined by atomic absorption spectrophotometry (SIMAA 6100, PerkinElmer, Waltham, MA, USA). The transferrin saturation percentage (Sat %) was determined using the following formula: Sat (%) = ((Serum iron/TIBC) × 100).

2.4. Content of Iron, Copper, and Zinc in Pork and Offal

Pigs were slaughtered by exsanguination on day 60 after being rendered unconscious using an anesthesia protocol, including an intramuscular injection of 2 mg/kg of azaperone and 5 mg/kg of ketamine. Then, a professional butcher identified and dissected the main pork cuts (Figure 1 and Table 2), which were deboned. From each meat cut (2 samples per hemicanal from the same animal), random samples of each muscle group measuring 1 × 1 × 1 cm³ were selected and then frozen at −18 °C. In addition, the liver, spleen, kidneys, heart, brain, and pancreas were collected. Iron, copper, and zinc contents were determined in meat and offal by atomic absorption spectrophotometry after acid digestion [23]. Non-heme iron content was determined by a microanalysis method of non-heme iron in animal tissues [25]. Briefly, tissues were homogenized in deionized water at 1:10 w/v. Then, tissue homogenates and protein precipitation solution (1 N HCl and 10% trichloroacetic acid) were combined in micro-centrifuge tubes and placed in a 95 °C heating block for 1 h. The tubes and their contents were cooled in water at room temperature for 2 min, vortexed, and then centrifuged at 16,000 × g for 10 min. Supernatant aliquots were mixed with chromogen solution (0.508 mmol/L ferrozine, 1.5 mol/L
sodium acetate, and thioglycolic acid at 1.5% v/v in deionized water). After 30 min at room temperature, absorbance was measured with a spectrophotometer (SIMAA 6100, PerkinElmer, Waltham, MA, USA). Heme iron content was calculated as the difference of total iron and non-heme iron.

Figure 1. Diagram of American pork cuts (https://animalscience.unl.edu/pork-meat-identification, accessed on 1 February 2021).

Table 2. Retail names of pork cuts.

| Cuts            | Retail Names                                                      |
|-----------------|-------------------------------------------------------------------|
| Shoulder butt   | Boston shoulder, pork butt, Boston butt                          |
| Picnic shoulder | Shoulder arm picnic, picnic shoulder, fresh picnic, picnic roast  |
| Shoulder hock   | Front hock                                                        |
| Center loin     | Loin end chop, center loin chop, sirloin chop                     |
| Short plate     | Short ribs                                                       |
| Spare ribs      | Pork ribs, baby back ribs                                        |
| Tenderloin      | Pork filet, pork tender                                          |
| Ham             | Fresh ham, whole ham                                             |
| Half ham        | Shank end, ham hock                                              |

2.5. Statistical Analysis

The normality of the data was confirmed by a Shapiro–Wilk test ($p > 0.05$). Pre- and post-supplementation changes of hematological biomarkers and serum microminerals were analyzed with a two-way ANOVA for repeated measures using the GLM procedure of SAS (version 9.0; SAS Institute Inc., Cary, NC, USA). Mean values are presented as
least square means adjusted by Tukey’s test. The α level used for the determination of significance was 0.05. The following mathematical model was used (1):

\[ Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \epsilon_{ijk} \]  

(1)

where \( Y \) is the hematological biomarkers or serum microminerals, \( \mu \) is the general mean of all observations, \( \alpha \) is the effect of treatment (control or supplemental group), \( \beta \) is the effect of the initial or final time of evaluations (0 and 60 days), \( \gamma \) is the interaction (treatment x time), and \( \epsilon \) is the random error.

Iron, zinc, and copper concentrations in pork samples were compared by a Student’s \( t \)-test (\( p < 0.05 \)), using Statgraphics Plus 5 software (Statistical Graphics Corp, Rockville, MA, USA).

3. Results

3.1. Diet and Performance Parameters

The diet presented in Table 1 describes the macro- and micronutrient requirements of pigs, specifically iron, copper, and zinc. At the beginning of the study, no difference in live weight between the control (15.6 ± 2.1 kg) and iron supplemental (14.2 ± 1.7 kg) groups was observed. At the end of the study, no differences were observed for the live weight (44.0 ± 3.6 and 42.0 ± 2.5 kg) and mean weight gain (0.474 ± 0.028 and 0.464 ± 0.046 kg) for control and iron supplemental pigs, respectively.

3.2. Hematological Parameters and Serum Micromineral Concentration

At the beginning of the study (day 0), hematological parameters and serum concentrations of iron, copper, and zinc were similar in both groups (Table 3). For hematological parameters, after iron supplementation (day 60), both groups showed an increase in hemoglobin, hematocrit, and transferrin saturation (Table 3). However, the iron supplemental group showed hemoglobin and hematocrit values that were significantly higher than the control group (Table 3). No significant differences were found in serum concentrations of iron and zinc. Serum copper levels increased in both groups as a consequence of time but not as an effect of the treatment (Table 3).

### Table 3

| Parameters | Day 0 | Day 60 | Effects (p-Value) |
|------------|-------|--------|-------------------|
|            | Fe500 | Fe3000 | SEM | Fe500 | Fe3000 | SEM | Treatment | Time | Interaction |
| Hb (g/dL)  | 10.9  | 12.1   | 0.292 | 13.6  | 15.0   | 0.492 | 0.006 | <0.001 | 0.176 |
| Ht (%)     | 35.1  | 37.3   | 0.080 | 40.2  | 45.2   | 0.592 | 0.010 | <0.001 | 0.167 |
| MCV (fL)   | 53.9  | 53.8   | 0.006 | 54.3  | 53.4   | 0.020 | 0.310 | 0.432 | 0.223 |
| Sat (%)    | 15.7  | 17.9   | 0.232 | 22.8  | 30.3   | 0.649 | 0.218 | 0.013 | 0.198 |
| Fe (µg/dL) | 85.6  | 93.0   | 0.691 | 116.2 | 127.9  | 0.206 | 0.114 | 0.132 | 0.221 |
| Zn (µg/dL) | 137.0 | 139.4  | 0.255 | 121.9 | 122.2  | 0.018 | 0.098 | 0.101 | 0.296 |
| Cu (µg/dL) | 114.0 | 115.7  | 0.223 | 142.2 | 155.4  | 0.586 | 0.172 | <0.001 | 0.098 |

Control (Fe500) and iron supplemental (Fe3000) groups. Hb: hemoglobin, Ht: hematocrit, MCV: medium corpuscular volume, Sat%: transferrin saturation percentage.

3.3. Content of Total Iron, Heme Iron, Copper, and Zinc in Pork

Table 4 shows the total iron, heme iron, copper, and zinc contents after oral iron supplementation (day 60) in the main American pork cuts. Total iron and heme iron contents were similar among the control and iron supplemental groups. Similar to those described for iron, no effects of iron supplementation on copper content in all pork cuts were observed. Importantly, oral iron supplementation had a significant effect of decreasing zinc content in all pork cuts. Zinc content was reduced by 32% in samples of tenderloin and by 55% in sparerib cuts.
Table 4. Effect of oral iron (Fe) supplementation on total Fe, heme Fe, copper (Cu), and zinc (Zn) contents in cuts of pork (mg/100 g).

| Cuts            | Fe500 | Fe3000 | SEM  | p-Value |
|-----------------|-------|--------|------|---------|
| Picnic shoulder | 0.7   | 0.7    | 0.040| 0.769   |
| Spare ribs      | 0.6   | 0.6    | 0.033| 0.860   |
| Shoulder hock   | 0.9   | 0.9    | 0.146| 0.654   |
| Short plate     | 0.9   | 1.0    | 0.043| 0.365   |
| Ham             | 0.5   | 0.5    | 0.092| 0.718   |
| Half ham        | 0.8   | 0.9    | 0.048| 0.140   |
| Tenderloin      | 0.9   | 1.0    | 0.049| 0.331   |
| Shoulder butt   | 1.0   | 0.9    | 0.024| 0.331   |
| Center loin     | 0.5   | 0.5    | 0.088| 0.810   |

| Cuts            | Fe500 | Fe3000 | SEM  | p-Value |
|-----------------|-------|--------|------|---------|
| Picnic shoulder | 0.3   | 0.3    | 0.008| 0.891   |
| Spare ribs      | 0.3   | 0.3    | 0.047| 0.750   |
| Shoulder hock   | 0.5   | 0.5    | 0.008| 0.932   |
| Short plate     | 0.5   | 0.6    | 0.037| 0.393   |
| Ham             | 0.2   | 0.3    | 0.041| 0.143   |
| Half ham        | 0.5   | 0.5    | 0.037| 0.114   |
| Tenderloin      | 0.5   | 0.5    | 0.032| 0.539   |
| Shoulder butt   | 0.6   | 0.5    | 0.039| 0.808   |
| Center loin     | 0.3   | 0.2    | 0.040| 0.518   |

| Cuts            | Cu    | Cu    | Cu   | Cu   |
|-----------------|-------|-------|------|------|
| Picnic shoulder | 0.1   | 0.1   | 0.014| 0.754|
| Spare ribs      | 0.1   | 0.1   | 0.024| 0.212|
| Shoulder hock   | 0.1   | 0.1   | 0.002| 0.692|
| Short plate     | 0.1   | 0.1   | 0.005| 0.005|
| Ham             | 0.1   | 0.1   | 0.005| 0.684|
| Half ham        | 0.1   | 0.1   | 0.004| 0.452|
| Tenderloin      | 0.1   | 0.1   | 0.019| 0.096|
| Shoulder butt   | 0.1   | 0.1   | 0.008| 0.096|
| Center loin     | 0.1   | 0.1   | 0.019| 0.066|

| Cuts            | Zn    | Zn    | Zn   | Zn   |
|-----------------|-------|-------|------|------|
| Picnic shoulder | 1.8   | 1.0   | 0.278| 0.001|
| Spare ribs      | 2.0   | 0.9   | 0.146| <0.001|
| Shoulder hock   | 2.5   | 1.5   | 0.291| 0.002|
| Short plate     | 3.0   | 1.4   | 0.273| <0.001|
| Ham             | 1.5   | 0.7   | 0.174| <0.001|
| Half ham        | 2.8   | 1.4   | 0.122| <0.001|
| Tenderloin      | 1.9   | 1.3   | 0.206| 0.001|
| Shoulder butt   | 2.9   | 1.5   | 0.196| <0.001|
| Center loin     | 1.1   | 0.6   | 0.104| <0.001|

Control (Fe500) and supplemented (Fe3000) groups.

3.4. Content of Total Iron, Non-Heme Iron, Copper, and Zinc in Pork Offal

Table 5 shows total iron, non-heme iron, zinc, and copper contents in pork offal after supplementation (day 60) from control and iron supplemental groups. The main effect of iron supplementation was an important increase in total iron and non-heme iron contents in most offal, with the exceptions of the heart and brain. The increase in total iron in the pancreas and spleen was close to double, and was 66% for the liver. It was observed that the liver and pancreas almost doubled their non-heme iron content post-supplementation, and the spleen increased its non-heme iron content 3.3-fold. The kidney had a lower increase in non-heme iron just as it had a lower total iron. The heart and brain maintained similar non-heme iron concentrations at the end of the supplementation. Finally, iron supplementation had no effect on the copper and zinc contents in any offal.
Table 5. Effect of oral iron (Fe) supplementation on total Fe, non-heme Fe, copper (Cu), and zinc (Zn) concentrations in pork offal (mg/100 g).

| Offal   | Fe500  | Fe3000 | SEM   | p-Value |
|---------|--------|--------|-------|---------|
|         | Total Fe |        |       |         |
| Liver   | 20.6  | 45.6  | 6.880 | 0.003   |
| Spleen  | 14.9  | 31.3  | 2.438 | <0.001  |
| Kidney  | 5.7   | 7.6   | 0.362 | 0.032   |
| Heart   | 3.8   | 4.0   | 0.269 | 0.666   |
| Brain   | 1.0   | 1.1   | 0.191 | 0.675   |
| Pancreas| 1.3   | 2.2   | 0.126 | <0.001  |

| Offal   | Non-Heme Fe |        |       |         |
|---------|             |        |       |         |
| Liver   | 14.0  | 32.3  | 6.688 | 0.022   |
| Spleen  | 6.2   | 20.3  | 1.834 | <0.001  |
| Kidney  | 3.8   | 4.5   | 0.105 | 0.006   |
| Heart   | 1.4   | 1.5   | 0.227 | 0.608   |
| Brain   | 0.6   | 0.6   | 0.040 | 0.480   |
| Pancreas| 0.7   | 1.1   | 0.050 | <0.001  |

| Offal   | Cu      |        |       |         |
|---------|---------|--------|-------|---------|
| Liver   | 0.6    | 0.6   | 0.020 | 0.246   |
| Spleen  | 0.1    | 0.1   | 0.018 | 0.460   |
| Kidney  | 0.7    | 0.6   | 0.089 | 0.304   |
| Heart   | 0.3    | 0.3   | 0.019 | 0.323   |
| Brain   | 0.3    | 0.3   | 0.028 | 0.825   |
| Pancreas| 0.1    | 0.2   | 0.037 | 0.317   |

| Offal   | Zn      |        |       |         |
|---------|---------|--------|-------|---------|
| Liver   | 8.2    | 8.3   | 0.425 | 0.855   |
| Spleen  | 2.3    | 2.1   | 0.060 | 0.106   |
| Kidney  | 2.5    | 2.5   | 0.140 | 0.856   |
| Heart   | 1.6    | 1.6   | 0.049 | 0.200   |
| Brain   | 1.1    | 1.1   | 0.079 | 0.728   |
| Pancreas| 3.8    | 4.7   | 0.771 | 0.305   |

Control (Fe500) and supplemental (Fe3000) groups.

4. Discussion

The harmful effect of overdoses of orally and parenterally delivered iron on the health of pigs has been extensively studied [14–17]. However, the effect of high oral iron doses on the content of microminerals in pork cuts has been poorly documented. This issue is very relevant since meat is one of the main sources of iron (especially of heme origin) and zinc for humans [5–7].

After oral supplementation with a dose of 3000 ppm iron for 60 days, the pigs showed an increase in some hematological parameters, such as hemoglobin, hematocrit, and transferrin saturation, which is expected in studies of this type and has been described in other studies of oral iron supplementation in pigs of different ages [11,14,26]. The increase in transferrin saturation is explained by the fact that the iron consumed in the diet was absorbed [27]. Approximately 60–70% of body iron is found in the blood as part of erythrocytes, triggering an increase in hemoglobin and hematocrit. This also explains why serum iron did not increase post-supplementation, since excess iron is stored as deposits [27,28] or excreted in urine and feces if iron needs are satisfied [15].

Hepcidin is the main peptide regulator of iron homeostasis in humans and pigs. It has been demonstrated that pigs receiving high doses of iron express greater amounts of hepcidin, which blocks the outflow of iron from enterocytes or macrophages into circulation [16]. This also could explain why no differences in serum iron levels were observed.
Iron supplementation also had no effect on serum copper and zinc concentrations since the diet covered the requirements of both microminerals. Differences in serum levels of copper and zinc following iron supplementation have not been reported [26].

The main effect of high-dose iron supplementation was a considerable reduction in zinc content in all the pork cuts analyzed. In contrast, total iron, heme iron, and copper were found to be within the normal ranges for pork meat (0.5 to 1.2 mg/100 g of iron and 0.08 to 0.15 mg/100 g of copper). Heme iron content fluctuated from 42% to 59% for the control group and from 46% to 60% for the supplemental group, which is considered to be within the normal range for pork meat [7,29,30]. Oral iron supplementation did not affect either total iron or heme iron in meat cuts, which is in agreement with other authors [31,32].

In this study, the zinc content in all pork cuts for the iron supplemental group was below the range considered normal (1.0 to 3.2 mg/100 g) [7,29]. A possible explanation is the antagonistic effect of iron, when consumed in high concentrations, on zinc absorption [33,34], which has been reported in humans [35,36] and pigs [37]. The reduction in zinc absorption may be due to iron-and-zinc-sharing receptors and transporters that participate in their absorption at the small intestinal level, such as the proteins in the ZIP family (ZIP1−14) [38] and the divalent metal transporter 1 (DMT1). Iron reduces zinc entry into DMT1 by up to 50% when the concentrations of these metals are in a 10:1 (iron:zinc) ratio [34]. It has also been observed that hepcidin reduces the expression of DMT1 [35,39], further reducing zinc absorption. Notably, low serum levels of zinc were not found after iron supplementation, but zinc concentrations did decrease dramatically in all pork cuts. This finding could be explained by the fact that muscle tissue is a primary zinc-depositing tissue [27].

Although this was an exploratory study, our findings could contribute to human nutrition because of the high prevalence of zinc deficiency globally [3]. Zinc has been called a “problem” nutrient by the World Health Organization (WHO) because consuming the optimal amount from food is difficult without fortification [40]. However, the experts have indicated that zinc is not necessarily a problem nutrient when meat or some viscera, such as the liver, are consumed regularly [41,42]. As meat is a very important source of zinc, excessive iron supplementation should be seriously considered before implementation as a regular animal husbandry practice. To avoid zinc depletion in pork cuts, it is recommended to follow the iron supplementation recommendations of the National Research Council [22]. Additionally, future studies are needed to establish the maximum doses that can be delivered in iron supplements or in the diet of pigs that do not affect zinc deposition in muscles.

The copper content of pork cuts was similar between groups before and after supplementation, and correspond with ranges previously reported in the literature [7,29]. The copper content of pork was not affected by excessive iron supplementation, possibly because this mineral is primarily captured by the copper transporter enzyme, whereas the DMT1 plays a less important role in this process [34]. Unlike zinc, muscle tissue is not the main site of copper deposition, so it is not a good indicator of copper deficiency [27].

Finally, only iron increased in pork offal after supplementation. This effect was expected for the liver, spleen, and kidney as they are iron reservoir organs [27,43]. Other studies have also reported that iron supplementation increases iron reserves, especially in the liver [15,44]. Fang et al. [45] reported that iron glycine chelate supplementation almost doubled iron concentration in the liver and increased iron concentration by close to 30% in the kidneys. Iron supplementation had no effect on the content of iron in the brain and heart as they are organs that have little relation to iron metabolism.

5. Conclusions

Excessive iron supplementation of 3000 ppm decreased the zinc content in pork cuts by 32–55%, whereas total iron, heme iron, and copper contents were not affected. Additionally, supplementation led to the doubling of iron content in the liver, spleen, and pancreas, while the content of zinc and copper in offal remained unaltered. Zinc is a necessary mineral
and is most readily consumed via meat. Excess iron supplementation can decrease zinc levels in pork meat cuts, which can negatively impact consumers' nutrition. Although this study was exploratory with a small sample size, the finding of the dramatic reduction in zinc content in pork cuts due to excessive iron supplementation is important to consider for future studies, as most intensive pork farms perform iron supplementation practices without frequent monitoring.

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**References**

1. Prohaska, J.R. Impact of copper deficiency in humans. *Ann. N. Y. Acad. Sci.* 2014, 1314, 1–5. [CrossRef]
2. Carpenter, C.; Mahoney, A. Contributions of heme and nonheme iron to human nutrition. *Crit. Rev. Food Sci. Nutr.* 1992, 31, 333–367. [CrossRef]
3. Hambidge, M. Human zinc deficiency. *J. Nutr.* 2000, 130, 1344–1349. [CrossRef] [PubMed]
4. World Health Organization (WHO). *The Global Prevalence of Anaemia in 2011*; WHO: Geneva, Switzerland, 2015; pp. 1–43. ISBN 9789241564960.
5. Geissler, C.; Singh, M. Iron, meat and health. *Nutrients* 2011, 3, 283–316. [CrossRef] [PubMed]
6. Pereira, P.; Vicente, A. Meat nutritional composition and nutritive role in the human diet. *Meat Sci.* 2013, 93, 586–592. [CrossRef] [PubMed]
7. Lombardi-Boccia, G.; Martinez-Dominguez, B.; Aguzzi, A. Total heme and non-heme iron in raw and cooked meats. *J. Food Sci.* 2002, 67, 1738–1741. [CrossRef]
8. Perri, A.; Friendship, R.; Harding, J. An investigation of iron deficiency and anemia in piglets and the effect of iron status at weaning on post-weaning performance. *J. Swine Health Prod.* 2016, 24, 10–20.
9. Bowlus, C. The role in T cell development and autoimmunity. *Autoimmun. Rev.* 2003, 2, 73–78. [CrossRef]
10. Pedersen, S.; Saeed, I.; Friis, H.; Michaelesen, K. Effect of iron deficiency on Trichuris suis and Ascaris suum infections in pigs. *Parasitol. Res.* 2010, 107, 589–598. [CrossRef] [PubMed]
11. Antileo, R.; Figueroa, J.; Valenzuela, C. Characterization of a novel encapsulated oral iron supplement to prevent iron–deficiency anemia in neonatal piglets. *J. Anim. Sci.* 2016, 94, 157–160. [CrossRef]
12. Churio, O.; Durán, E.; Guzmán-Pino, S.; Valenzuela, C. Use of encapsulation technology to improve the efficiency of an iron oral supplement to prevent anemia in suckling pigs. *Animals* 2019, 9, 1. [CrossRef]
13. Staroñ, R.; Lipiñski, P.; Lenartowicz, M.; Bednarz, A.; Gajowiak, A.; Smuda, E.; Krzeptowski, W.; Pieszka, M.; Korolonek, T.; Hamza, I.; et al. Dietary hemoglobin rescues young piglets from severe iron deficiency anemia: Duodenal expression profile of genes involved in heme iron absorption. *PLoS ONE* 2017, 12, e0181117. [CrossRef] [PubMed]
14. Lipiñski, P.; Starzyñski, R.; Canonne-Hergaux, F.; Tudek, B.; Olrlski, R.; Kowalczyk, P.; Dzianan, T.; Thibaudeau, O.; Gralak, M.; Smuda, E.; et al. Benefits and risks of iron supplementation in anemic neonatal pigs. *Am. J. Pathol.* 2010, 177, 1233–1243. [CrossRef]
15. Lee, S.; Shinde, P.; Choi, J.; Park, M.; Ohh, S.; Kwon, I.; Chae, B. Effects of dietary iron levels on growth performance, hematological status, liver mineral concentration, fecal microflora, and diarrhea incidence in weaning pigs. *Biol. Trace Elem. Res.* 2008, 126, 57–68. [CrossRef]
16. Starzyñski, R.; Laarakkers, C.; Tjalsma, H.; Swinkels, D.; Pieszka, M.; Stys, A.; Mickiewicz, M.; Lipiñski, P. Iron supplementation in suckling piglets: How to correct iron deficiency anemia without affecting plasma hepcidin levels. *PLoS ONE* 2013, 8, e64022. [CrossRef]
17. Ji, P.; Lönnerdal, B.; Kim, K.; Jinno, C. Iron over supplementation causes hippocampal iron overloading and impairs social novelty recognition in nursing piglets. *J. Nutr.* 2019, 149, 398–405. [CrossRef] [PubMed]
