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By

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ABSTRACT

EFFECT OF ZINC ON THE GROWTH, DEVELOPMENT AND REPRODUCTION OF GILTS

By

Gretchen Myers Hill

The effects of several levels of dietary zinc on growing and developing gilts, their reproduction and offspring were studied. Gilts weighing 30 kg were provided with 0, 50, 500 or 5000 ppm additional dietary zinc from zinc oxide and were continued on their respective treatments through two parities. Weight and feed consumption were not affected during the growth and development phases of the study, but gilts on the highest supplemental treatment weighed less than the other gilts when killed. Gilts fed the 5000 ppm additional zinc had significantly higher serum alkaline phosphatase activity and serum zinc levels and significantly lower serum copper concentrations during the entire study and weaned fewer and smaller pigs than did sows on the other treatments. However, sows receiving no additional zinc had a higher number of abnormal pigs per litter than sows on the other treatments. Sows receiving 500 ppm supplemental zinc had fewer abnormal pigs than sows receiving 50 or 5000 ppm additional zinc.

The concentration of zinc in the sow's liver increased significantly and copper decreased as dietary level of zinc increased. Elevated renal copper and zinc concentrations were found in sows fed the highest level of zinc supplementation.

Pigs from sows fed 5000 ppm additional zinc had heavier liver, heart, thyroid and adrenal weight relative to their body weight than did pigs from sows on the other treatments. Pigs from first and second parity sows on the highest zinc supplementation level had higher iron

stores in the liver, higher zinc concentrations in the liver, kidney and pancreas, and higher copper levels in the kidney compared to pigs from sows on the other treatments. However, copper concentrations in the liver, heart, pancreas, esophagus, aorta and testes were reduced in pigs from sows on the 5000 ppm zinc treatment. In pigs from first parity sows, calcium in the liver was higher for pigs whose dams received 5000 ppm zinc compared to pigs from sows on all other treatments and the manganese level was higher compared to pigs from sows receiving 50 or 500 ppm additional zinc. Pigs at one day of age from sows on the 0, 50 or 500 ppm treatment had lower hepatic phosphorus and zinc concentrations than pigs from sows on the same treatment at 21 days of age. The reverse was true for pigs whose dams received 5000 ppm zinc.

Colostrum from sows fed 5000 ppm additional zinc contained less copper and phosphorus than milk from sows on the other treatments. Copper and zinc concentrations were reduced in the first, second and third week milk from sows fed 0, 50 or 500 ppm additional zinc compared to colostrum, but only zinc was reduced in milk from sows supplemented with 5000 ppm zinc compared to colostrum. Calcium concentrations were increased in all milk for all treatments compared to colostrum, and magnesium was increased in first, second and third week milk for sows fed 0, 50 or 500 ppm zinc and in second and third week milk for sows fed 5000 ppm zinc compared to colostrum. Phosphorus was higher in second week milk from sows fed 0 or 500 ppm zinc and higher in third week milk for sows fed 5000 ppm zinc compared to colostrum. The iron concentration in first week milk from the second parity was increased compared to that from the first parity for all treatments. Magnesium was reduced in second parity colostrum compared to first parity for all treatments except the unsupplemented group.

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SECTION I

Effect of Dietary Zinc on the Growth and Development of the Gilt

INTRODUCTION

The characteristics of a zinc deficiency in the growing rat, chick, pig and other non-ruminants are well documented in the literature. Conversely, the effects of high levels of zinc and attempts to delineate toxic zinc levels have been studied extensively in the rat and chick, but not in the pig. The known interactions of zinc with other dietary constituents such as protein source and level (McCall et al., 196la,b; Smith et al., 1962) and level of calcium (Stewart and Magee, 1964; Hsu et al., 1975) present in the diet which affect the zinc bioavailability (O'Dell, 1969) are especially important when studying high levels of zinc. Ammerman and Miller (1972) note that a critical evaluation of the bioavailability of different inorganic sources of zinc has only been carried out with poultry. From work in our lab (Hill et al., 1980) it appears that zinc carbonate is more highly available to the pig than zinc oxide. Thus, it is difficult to compare studies characterizing zinc's effects when different dietary protein levels and sources, mineral concentrations and zinc sources are utilized for varying periods of time.

Since the zinc status of a rat has been shown to influence the normalcy of a pregnancy and resulting offspring (Hurley and Swenerton, 1966), it was hypothesized that the same might be true for swine. Also, Mertz (1977) noted that the concept of saturation, which is exemplified by many element-dependent biological functions reaching a plateau with increasing levels of supplementation, is helpful in both

animal and human nutrition to establish normal values of growth and productivity to correlate with dietary intake. Therefore, our study was designed to assess the affects of several levels of supplemental zinc from one zinc source on the growth, development and reproduction of gilts fed a corn-soybean meal diet. The results of the growing and developing stages will be reported herein.

Experimental Procedure

Experimental animals. Sixty crossbred (Hampshire, Duroc, Yorkshire, Landrace) and purebred Yorkshire gilts initially averaging 30 kg body weight were allotted by sire into four treatment groups and blocked by the date they were farrowed into three blocks. The gilts were housed in a total confinement facility with slotted floors, cast-iron automatic waterers and wood/non-galvanized metal self-feeders. The pens were 4.27 by 1.21 meters during the grower phase and 4.87 by 1.21 meters until the gilts reached approximately 100 kg of body weight. At this time, they were moved from the total confinement facility to dirt or concrete lots.

A basal corn-soybean meal diet (grower) which met all NRC (1979) recommended dietary allowances was fed ad libitum until the lightest animals reached approximately 60 kg (table 1). The developer diet (table 1) was fed for the remainder of the study. After reaching approximately 100 kg body weight, the gilts were limit fed 1.75 kg to 2.75 kg of feed per day depending on climatic conditions. Water was available ad libitum throughout the study. The four dietary treatments were 0, 50, 500 or 5000 ppm of zinc added to the basal diet from feed grade zinc oxide (table 2). Table 3 contains the results of the laboratory analyses of the diets.

Table 1. Basal corn-soybean meal diet

Ingredient	Internat'l ref. no.	Grower & lactation %	Developer & gestation %
Ground shelled corn	4-02-992	78.4	83.9
Dehulled soybean meal	5-04-612	18.0	12.5
Dicalcium phosphate	6-01-080	.9	1.2
Calcium carbonate	6-01-069	1.2	.9
Salt		.5	.5
MSU vitamin-trace mineral premix[a]		.5	.5
Vitamin E-Se premix[b]		.5	.5

[a]Supplying the following vitamins and trace elements per kilogram of diet: vitamin A, 3300 IU; vitamin D, 660 IU; menadione sodium bisulfite, 2.2 mg; riboflavin, 3.3 mg; niacin, 17.6 mg; d-pantothenic acid, 13.2 mg; vitamin B_{12}, 19.8 µg; iron, 60 mg; copper, 10 mg; iodine, 2.8 mg; manganese, 37 mg.

[b]Supplying 17 IU of vitamin E and 100 µg of selenium per kilogram of diet.

Table 2. Dietary treatment and zinc content of diet by analysis

Treatment	Grower & lactation ppm, Zn	Developer & gestation ppm, Zn
Basal	34.7	37.1
Basal + 50 ppm Zn[a]	86.8	88.3
Basal + 500 ppm Zn[a]	525.0	550.0
Basal + 5000 ppm Zn[a]	5060.0	4992.0

[a]Zn provided as ZnO.

Table 3. Mineral content of the diets by chemical analysis

	Element						
Treatment	Cu,ppm	Fe,ppm	Ca,%	Mg,%	Mn,ppm	P,%	Se,ppm
Basal	14.7	172	.71	.16	65.2	.45	.16
+50 ppm Zn	15.9	205	.68	.15	61.8	.46	.15
+500 ppm Zn	15.7	199	.71	.16	66.8	.48	.15
+5000 ppm Zn	17.3	255	.66	.15	99.1	.49	.15

Weighing, blood sampling and analysis. The gilts were weighed individually and group feed consumption was recorded bi-weekly. Blood samples were obtained from the anterior vena cava every four weeks, and serum was stored at -20°C until analyzed. Serum alkaline phosphatase (EC 3.1.3.1) activity was assessed by Sigma's colorimetric procedure (Sigma Chemical Company, St. Louis, MO.) which is based on the Bessey-Lowry-Brock method and utilizes p-nitrophenyl phosphate in a glycine buffer as the substrate. Serum was diluted 1:7 with deionized-distilled water for determination of copper and zinc by atomic absorption spectrophotometry (IL-453, Instrumentation Laboratory, Lexington, MA.).

Statistical analyses. A modified version of Kolmogorov-Smirnov D-statistic was utilized to test for the probability of nonnormality of distribution. Because the serum alkaline phosphatase and zinc data were distinctly nonnormal, a natural logarithm transformation was utilized to ensure near normality of distribution for the transformed variables (Gill, 1978). Because the natural log of zero is indeterminate and the natural log of one is zero, one was added to each observation before the observation was converted to natural log value. Analysis of variance was performed using the General Linear Models procedure of the Statistical Analysis System maintained at Wayne State University. A procedure involving Bonferroni t statistics was utilized for comparisons among means (Gill, 1978). Because the animals were assigned to dietary treatments, grouped by date farrowed into blocks and measured for trend at four sampling times, a split-plot design was utilized. This design allows for the separation of random error into variation among and variation within subjects.

Results and Discussion

Split-plot design. Analysis of variance showed that sire did not affect the results, but there was a significant interaction between treatment X block X time, thus indicating nonparallel trends in response over time. Therefore, comparisons of treatments within blocks at each sampling time and comparisons of means from each sampling time within treatment and block were made. It is established within the swine industry that climatic conditions affect gain and feed efficiency because of an animal's need to maintain body temperature when it differs from ambient temperature (Mount, 1975; Fuquay, 1981). Since the animals within each block were begun on the experiment during the eighth, tenth and twelfth months of the year, their physiological state varied in the different seasons. Perhaps this is why block interacts with treatment and time.

After the natural logarithm transformation of the serum alkaline phosphatase and zinc data was completed, a modified version of the Kolmogorov-Smirnov D-statistic was utilized to test for the probability of nonnormality. The residuals of the log transformed serum alkaline phosphatase and zinc data were considered to be normally distributed. Although the accuracy of the probability statements is improved, natural logarithm transformed data are difficult to interpret. Therefore, the mean of the original data is also provided in the tables even though the natural log transformed values were utilized in the statistical analysis. The residuals of the variables body weight and serum copper were nonnormal at a lesser probability than the residuals of the natural log transformation and therefore, non-transformed data were analyzed.

Effect of treatment within block and sampling period on growth parameters. Treatment within block did not affect weight gain or feed efficiency although there was a trend for gilts receiving 5000 ppm added zinc to weigh less after 20 weeks (tables 4 and 5). Brink et al. (1959) reported that 2000 ppm of zinc from zinc carbonate depressed gain and feed consumption. These parameters exhibited an even greater depression when 4000 ppm was fed; animals receiving 8000 ppm lost weight. Cox and Hale (1962) did not observe any effects during a 69 day trial on the performance of pigs fed 2000 or 4000 ppm added zinc from zinc oxide. Our data support their findings and suggest that 5000 ppm zinc from zinc oxide can be tolerated without adverse effects on performance. The difference in tolerable levels when compared to the studies by Brink et al. (1959) may be due to zinc source. After reviewing the literature, Ammerman and Miller (1972) reported that zinc carbonate and oxide are both quite available to the chick. There is no information comparing zinc sources fed to swine. In a small study (Hill et al., 1980), we found serum alkaline phosphatase and serum zinc levels to be one and a half to two times higher in pigs fed zinc carbonate than in those fed zinc oxide. Hsu et al. (1975) found growth depressed in 7.5 kg pigs fed .7% calcium and 4000 ppm zinc from zinc oxide compared to pigs fed 1.1% calcium with the same level and source of zinc. NRC (1979) recommends .8% calcium in the diet for pigs of this weight. Stewart and Magee (1964) utilizing rats found that zinc has an antagonistic effect on the normal deposition of calcium and phosphorus in bones and causes increased excretion of these elements. Also, calcium and phosphorus supplementation prevented the increased accumulation of zinc in the bone, perhaps by facilitating the removal

Table 4. Effect of treatment within blocks on body weight, kg

	Time, weeks											
	0			4			12			20		
Treatment	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
Block 1												
0 ppm	5	35.36	5.88	5	56.40	5.88	5	105.20	5.88	5	143.64	5.88
50 ppm	5	37.56	5.88	5	62.04	5.88	5	111.24	5.88	4	155.70	6.57
500 ppm	5	34.80	5.88	5	55.12	5.88	5	108.08	5.88	5	147.90	5.88
5000 ppm	5	37.92	5.88	5	62.24	5.88	5	107.16	5.88	5	140.64	5.88
Block 2												
0 ppm	5	31.24	5.88	5	52.40	5.88	5	100.40	5.88	5	138.56	5.88
50 ppm	5	33.12	5.88	5	57.12	5.88	5	102.24	5.88	4	138.08	6.57
500 ppm	5	30.68	5.88	5	54.12	5.88	5	100.84	5.88	5	130.36	5.88
5000 ppm	5	30.08	5.88	5	54.88	5.88	5	97.00	5.88	5	115.56	5.88
Block 3												
0 ppm	5	28.20	5.88	5	47.28	5.88	5	92.40	5.88	5	116.76	5.88
50 ppm	5	28.24	5.88	5	47.88	5.88	5	92.78	5.88	5	123.20	5.88
500 ppm	5	28.40	5.88	5	49.32	5.88	5	86.66	5.88	5	118.20	5.88
5000 ppm	5	28.56	5.88	5	51.38	5.88	5	87.46	5.88	5	103.90	5.88

Table 5. Feed intake and feed/gain by treatment and block with time

	kg feed 0-4 weeks[a]	F/G	kg feed 4-12 weeks[b]	F/G
Block 1				
0 ppm	327.7	3.12	813.6	3.33
50 ppm	334.1	2.73	878.6	3.57
500 ppm	338.6	3.33	931.4	3.52
5000 ppm	343.0	2.82	888.0	3.95
Block 2				
0 ppm	318.2	3.01	761.8	3.17
50 ppm	327.7	2.73	676.8	3.00
500 ppm	311.4	2.66	700.9	3.00
5000 ppm	297.7	2.40	666.4	3.16
Block 3				
0 ppm	312.7	3.27	732.5	3.24
50 ppm	324.1	3.30	736.8	3.28
500 ppm	327.7	3.13	665.5	3.56
5000 ppm	349.1	3.06	657.7	3.65

[a]Four week intake of 5 pigs.

[b]Eight week intake of 5 pigs.

of excess zinc before it is absorbed from the animal's body. The difficulty in comparing these studies is compounded because initial weights varied from 7.5 kg (Hsu et al., 1975), 12.7 kg (Cox and Hale, 1962), 16.4 kg (Brink et al., 1959) to 30 kg in this study. Miller et al. (1977) noted that the homeostatic control mechanism(s) with high zinc diets differ(s) with species, age and tissue.

Effect of treatment within block on serum alkaline phosphatase activity. After four weeks, serum alkaline phosphatase (EC 3.1.3.1) activity was elevated in all animals receiving 5000 ppm added zinc in all blocks (table 6). In block one, the activity of this enzyme was higher than the activity of animals receiving 0 ppm added zinc at all sampling times except 20 weeks. In block two, this enzyme's activity was lower in gilts being fed 50 ppm added zinc at 4, 12, and 20 weeks and those fed 500 ppm at 12 and 20 weeks compared to those receiving the highest zinc treatment. Gilts supplemented with 50 ppm zinc had the lowest serum alkaline phosphatase activity at four weeks, but the activity was lowest at 12 and 20 weeks in the serum of the unsupplemented gilts in block three. At 20 weeks, serum from 50 and 500 ppm gilts had a similar activity level which was higher than those receiving no added zinc but significantly lower ($P<.05$) than those receiving 5000 ppm zinc. Hsu et al. (1975) observed elevated serum alkaline phosphatase when 4000 ppm zinc was added to a diet containing .7% calcium, but their values were considerably lower than those reported herein. Long et al. (1965) found this enzyme to vary greatly both within and between litters of pigs at birth, 1, 7 and 14 days of age and to decrease in phosphatase activity after one day of age. Burch et al. (1975) reported that young pigs fed ad libitum a diet containing

Table 6. Effect of treatment within block on serum alkaline phosphatase activity, Sigma units/ml

	Time, weeks															
	0				4				12				20			
Treatment	N	Mean	Mean ln	SE	N	Mean	Mean ln	SE	N	Mean	Mean ln	SE	N	Mean	Mean ln	SE
Block 1																
0 ppm	5	1.62	.88[a]	.09	5	0.87	.61[a]	.09	5	0.79	.56[a]	.09	5	1.02	.69[a]	.09
50 ppm	5	1.94	.95	.09	5	1.44	.88	.09	5	1.55	.93	.09	4	1.23	.80	.10
500 ppm	5	2.44	1.15	.09	5	1.44	.88	.09	5	1.65	.97	.09	5	1.25	.81	.09
5000 ppm	5	3.19	1.37[b]	.09	5	2.45	1.23[b]	.09	5	3.04	1.37[b]	.09	5	1.89	1.05	.09
Block 2																
0 ppm	5	2.67	1.29[x]	.09	5	2.01	1.10	.09	5	1.05	.71[ya]	.09	5	1.82	1.03	.09
50 ppm	5	2.59	1.28[x]	.09	5	1.59	.95[a]	.09	5	1.34	.81[ya]	.09	4	1.74	.99[a]	.10
500 ppm	5	1.95	1.07	.09	5	2.40	1.22	.09	5	1.83	1.04[a]	.09	5	1.31	.83[a]	.09
5000 ppm	5	2.52	1.23[x]	.09	5	3.65	1.50[b]	.09	5	4.36	1.67[yb]	.09	5	3.41	1.45[b]	.09
Block 3																
0 ppm	5	2.27	1.18[x]	.09	5	1.91	1.03[x]	.09	5	1.35	.85[a]	.09	5	0.81	.59[ay]	.09
50 ppm	5	2.11	1.12	.09	5	1.74	.95[a]	.09	5	1.95	1.08	.09	5	1.62	.95[b]	.09
500 ppm	5	2.21	1.15	.09	5	2.20	1.13	.09	5	1.85	1.04	.09	5	1.42	.85[ab]	.09
5000 ppm	5	2.30	1.18	.09	5	3.14	1.41[b]	.09	5	3.88	1.57[b]	.09	5	3.29	1.45[c]	.09

[abc]Values with different superscripts in the same column within blocks are significantly different (P<.05).

[xyz]Values with different superscripts on the same line are significantly different (P<.05).

120 ppm zinc had serum alkaline phosphatase activity levels that did not differ from pigs fed a diet containing 7.3 ppm (59 vs 63 U/liter). Miller et al. (1968) reported that baby pigs fed 12 ppm had significantly lower serum alkaline phosphatase activity than pigs fed a diet containing 100 ppm zinc (.7 vs 9.1 Sigma units/ml). Since this enzyme requires zinc and is sensitive to a zinc deficiency, it has been proposed as an appropriate metalloprotein for diagnosis of a zinc deficiency (Danks, 1981).

Serum alkaline phosphatase is considered to be associated with osteoblastic and excessive osteolytic activity. Therefore, a decrease in activity in older healthy animals is expected and an increase in activity is expected when diseases such as osteoporosis, osteosclerosis, rickets, hepatic cirrhosis and hyperparathyroidism are present. Adeniyi and Heaton (1980) hypothesized that a zinc deficiency reduced the efficiency of operation and the amount of alkaline phosphatase in serum. No treatment means within block one were significantly different at any of the time periods. The phosphatase activity was significantly lower in the serum of gilts in block two fed 0 or 50 ppm zinc at 12 weeks but was significantly higher in gilts fed 5000 ppm zinc than initial activities for each respective treatment. Thus, gilts on this highest zinc treatment did not have decreased serum alkaline phosphatase activity with age. It can not be discerned from this study if the additional zinc consumed by these gilts stimulated the initiation of a larger amount of this enzyme or if increased osteolytic activity caused the increase. The serum from the unsupplemented gilts in block three had decreased activity at 20 weeks.

Effect of treatment within block on serum zinc. In the 5000 ppm zinc treatment of all blocks serum zinc was elevated after four weeks (table 7), reflecting the increased circulating zinc present in the body. This serum mineral was highest at 12 weeks and decreased, although not significantly, by 20 weeks. The values reported herein are not as high as those reported by Hsu et al. (1975), but values for the 0, 50 and 500 ppm treatment groups are similar to those reported by Miller et al. (1968) for the baby pig. In block 2 at 12 weeks, zinc in the serum from gilts fed 0 ppm zinc was lower than serum zinc from gilts fed the other dietary treatment. Gilts fed the highest treatment level had serum zinc levels higher than that of gilts fed 50 or 500 ppm. In block three, gilts fed the two lowest supplemental levels had lower serum zinc levels at 8 weeks but only the unsupplemented group was reduced by 12 weeks. Serum zinc increased with time in the highest supplemented treatment animals in blocks one and three and in 500 ppm treatment group in block three. Conversely, this mineral decreased with time in the serum of gilts fed 0, 50 or 500 ppm in block two and 0 or 50 ppm in block three. The pattern of serum zinc levels does not mirror that of alkaline phosphatase activity which might be expected if serum zinc above a certain threshold stimulated additional enzyme initiation.

Effect of treatment within block on serum copper. Copper in the serum of gilts in block one fed 5000 ppm supplemental zinc was depressed after 20 weeks of the study. At the end of four weeks, it was significantly lower ($P<.05$) in the highest zinc treatment group than in all other treatment groups in block two, and this pattern continued throughout the study. In block 3, serum copper was reduced at 4 weeks

Table 7. Effect of treatment within block on serum zinc, µg/dl

	Time, weeks															
	0				4				12				20			
Treatment	N	Mean	Mean ln	SE	N	Mean	Mean ln	SE	N	Mean	Mean ln	SE	N	Mean	Mean ln	SE
Block 1																
0 ppm	5	79.6	4.25	.12	5	51.2	3.92^{a}	.12	5	60.8	4.10^{a}	.12	5	63.2	4.15^{a}	.12
50 ppm	5	90.0	4.41	.12	5	45.2	3.82^{a}	.12	5	65.4	4.18^{a}	.12	4	62.3	4.14^{a}	.14
500 ppm	5	62.2	4.08	.12	5	79.8	4.37^{a}	.12	5	93.6	4.51^{a}	.12	5	65.4	4.19^{a}	.12
5000 ppm	5	66.4	4.08^{x}	.12	5	164.2	5.08^{b}	.12	5	246.2	5.49^{by}	.12	5	142.4	4.96^{b}	.12
Block 2																
0 ppm	5	97.4	4.56^{x}	.12	5	38.8	3.67^{ay}	.12	5	36.6	3.56^{ay}	.12	5	36.8	3.60^{ay}	.12
50 ppm	5	116.2	4.72^{x}	.12	5	44.4	3.80^{ay}	.12	5	68.6	4.23^{b}	.12	4	62.0	4.12^{a}	.14
500 ppm	5	121.6	4.79^{x}	.12	5	59.8	4.07^{ay}	.12	5	61.2	4.10^{by}	.12	5	57.4	4.06^{ay}	.12
5000 ppm	5	106.2	4.65	.12	5	220.2	5.40^{b}	.12	5	254.2	5.54^{c}	.12	5	156.0	5.05^{b}	.12
Block 3																
0 ppm	5	96.4	4.56^{x}	.12	5	43.2	3.67^{ay}	.12	5	38.2	3.55^{ay}	.12	5	52.8	3.97^{a}	.12
50 ppm	5	85.6	4.46	.12	5	51.8	3.93^{abx}	.12	5	114.6	4.74^{by}	.12	5	69.6	4.25^{a}	.12
500 ppm	5	60.6	4.12^{x}	.12	5	80.6	4.39^{b}	.12	5	124.8	4.83^{by}	.12	5	79.0	4.38^{a}	.12
5000 ppm	5	83.4	4.42^{x}	.12	5	258.2	5.54^{cy}	.12	5	281.8	5.64^{cy}	.12	5	217.2	5.34^{by}	.12

^{abc}Values with different superscripts in the same column within blocks are significantly different ($P<.05$).

^{xy}Values with different superscripts on the same line are significantly different ($P<.05$).

in 5000 vs 50 ppm treatments and 0 and 50 vs 5000 ppm at 20 weeks. Cox and Harris (1960) reported decreased plasma copper when rats were fed 4000 ppm zinc, but copper levels were restored to normal when 100 ppm of copper was fed with the elevated zinc. Ott et al. (1966b) found a significant linear effect in serum copper of calves when zinc was increased in the diet in increments of 400 from 100 ppm to 2100 ppm and serum copper was decreased from 1.1 μg/ml to .7 μg/ml. Ott et al. (1966a) also reported a decrease in the serum copper of lambs fed 2000 or 4000 ppm zinc compared to 0 or 500 ppm added zinc. VanCampen (1966) reported that when doses of ^{64}Cu and zinc were placed directly into in vivo ligated stomachs or duodenums the percent of the dose found in the blood was significantly lower if the zinc/copper ratio was 500 compared to ratios of 0 or 50. After evaluating isotope experiments, Magee and Matrone (1960) concluded that zinc interferes with copper metabolism by decreasing its utilization and increasing its excretion but has little effect on copper's absorption. Our study (table 8) shows that within the 5000 ppm treatment in each block there is a depression of serum copper with time. It is depressed after 12 and 20 weeks in block one, after 4, 12 and 20 weeks in block two and after 20 weeks in block three. This reduction of serum copper with time may reflect the amont of copper available for circulation, a decreasing amount of copper being absorbed or an increasing amount of copper being excreted. Since feed consumption also increased with time, the amount of available copper ingested would increase, but body size is not static. It appears that high levels of dietary zinc reduce serum copper in several species and that its level in serum continues to be reduced while the high level of zinc is fed.

Table 8. Effect of treatment within block on serum copper, µg/dl

	Time, weeks											
	0			4			12			20		
Treatment	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
Block 1												
0 ppm	5	204.6	13.6	5	193.6	13.6	5	175.4	13.6	5	194.2^{a}	13.6
50 ppm	5	178.0	13.6	5	196.8	13.6	5	165.2	13.6	4	181.8	15.2
500 ppm	5	206.8^{x}	13.6	5	135.2^{y}	13.6	5	186.6	13.6	5	179.6	13.6
5000 ppm	5	206.8^{x}	13.6	5	152.2	13.6	5	130.0^{y}	13.6	5	122.4^{by}	13.6
Block 2												
0 ppm	5	175.8	13.6	5	185.2^{a}	13.6	5	204.2^{a}	13.6	5	192.8^{a}	13.6
50 ppm	5	170.8	13.6	5	203.4^{a}	13.6	5	218.6^{a}	13.6	4	208.8^{a}	15.2
500 ppm	5	189.4	13.6	5	205.4^{a}	13.6	5	164.8^{a}	13.6	5	190.0^{a}	13.6
5000 ppm	5	200.4^{x}	13.6	5	88.8^{by}	13.6	5	62.8^{by}	13.6	5	80.8^{by}	13.6
Block 3												
0 ppm	5	217.2	13.6	5	228.4^{a}	13.6	5	175.4^{x}	13.6	5	258.6^{ay}	13.6
50 ppm	5	165.4	13.6	5	174.2	13.6	5	195.6	13.6	5	160.8^{a}	13.6
500 ppm	5	196.4	13.6	5	154.8	13.6	5	189.8	13.6	5	150.2	13.6
5000 ppm	5	177.8^{x}	13.6	5	119.4^{b}	13.6	5	138.6	13.6	5	86.6^{by}	13.6

^{ab}Values with different superscripts in the same column within block are significantly different ($P<.05$).

^{xy}Values with different superscripts on the same line are significantly different ($P<.05$).

In summary, 5000 ppm of zinc in the diet supplied from zinc oxide reduces serum copper but increases serum zinc and alkaline phosphatase activity progressively with time.

In general, serum alkaline phosphatase activity and zinc are lower when no additional zinc is added to the diet than if the diet is supplemented with 50 or 500 ppm. These parameters appear to plateau with the 50 and 500 ppm treatments, thus indicating a saturation. Serum copper level is not altered by these lower levels of supplementation.

SECTION II

Effect of Dietary Zinc Levels on Health and Reproductivity Through Two Parities

Introduction

The need for zinc in normal reproduction in both males and females has been studied extensively in rats. Zinc-deficient females have been observed to have abnormal estrous cycles (Swenerton and Hurley, 1968), resorption of implantation sites, malformed offspring and impaired parturition (Apgar, 1968, 1977; Hurley and Swenerton, 1966). Swenerton and Hurley (1980) have reported similar results with rhesus and bonnet monkeys.

In recent years, investigators have become more concerned about the zinc status of pregnant humans relative to a successful pregnancy and parturition (Bergmann, et al., 1980; Hunt et al., 1979; Sarram et al., 1969; Schechter et al., 1977; Shearer et al., 1979). Hoekstra et al. (1967) reported decreased liveability of pigs whose dams received a low zinc diet, and Wegger and Palludan (1977) found that gilts depleted of zinc in their last trimester had a longer gestation, an impaired parturition and offspring with low viability; abnormal skeletal ossification was often observed. The effect of long term feeding of high levels of zinc on females of any species and their reproductive performance has not been investigated.

Considering the importance of saturation in establishing normal values for productivity and maximum safe limits, our study was designed to assess the affects of several levels of supplemental zinc on the growth, development and reproduction (two parities) of gilts. The results of the reproductive phase of this study are reported herein.

Experimental Procedure

Experimental Animals. These animals are a part of a long term study to investigate the effects on the reproductive female of adding 0, 50, 500, or 5000 ppm zinc from zinc oxide to a corn-soybean diet. The results of the growth and development stages, details of the experimental procedure and information about dietary content are reported in a previous paper (Hill, et al., 1981). Gilts were field-mated between seven and eight months of age for the first parity. Sows were hand-mated for their second parity at the first estrus following weaning. Gilts/sows were moved by group to individual crates in the farrowing facility when gestational length was approximately 110 days for at least one of the gravid animals. After farrowing and weaning, sows were housed individually or by treatment group in a confinement facility with partial or total slats. If an animal were non-gravid after a minimum of three matings, were considered to be in too poor health to continue in the study, or had completed two parities, she was killed and tissues were collected. The developer diet was limit-fed during gestation (1.75 kg to 2.75 kg/day) until the sows entered the farrowing facility, and the grower diet was fed ad libitum during lactation (Hill et al., 1981, table 1).

Weighing, blood and tissue sampling and analyses. The gilts/sows were weighed every four months and prior to being killed. Blood samples were obtained from the anterior vena cava at 10 and 14 months of age. Serum was partitioned into two components for determining (1) copper and zinc and (2) serum alkaline phosphatase (SAP) and glutamic oxaloacetic transaminase (SGOT) activity. SGOT (EC 2.6.1.1) activity was assessed immediately after the bleeding of all animals by Sigma's

colorimetric procedure (Sigma Chemical Company, St. Louis, MO.) which utilizes aspartate-α-ketoglutarate as a substrate and 2,4-dinitrophenyl hydrazine for a color reagent. SAP (EC 3.1.3.1) activity was measured by Sigma's colorimetric procedure which is based on the Bessey-Lowry-Brock method and utilizes p-nitrophenyl phosphate in a glycine buffer as the substrate. Serum was stored at -20° C until analyzed and was diluted 1:7 with deionized-distilled water for determination of copper and zinc by atomic absorption spectrophotometry (IL-453, Instrumentation Laboratory, Lexington, MA). When the gilts/sows were killed, tissues were obtained and frozen at -20° C until analyzed. Uniform samples were cut from tissues, and duplicate samples were wet digested in a mixture of nitric and perchloric acids and diluted with deionized-distilled water as necessary for analyses by atomic absorption spectrophotometry. The hinge joints between the trochlea and capitulum of the humerus and the proximal ends of the ulna and radius respectively, were examined on each gilt/sow for abnormalities and evidence of erosion of the tissue. Each joint was scored on a scale from 0 (no evidence of erosion or change) to 5 (severe deterioration of the joint).

Statistical analyses. A modified version of Kolmogorov-Smirnov D-statistic was used to test for the probability of nonnormality. Because the (1) productivity parameters, (2) the zinc, copper and iron concentration in the tissues; and (3) the serum alkaline phosphatase activity and zinc concentration data were distinctly nonnormal, a natural logarithm transformation was utilized to ensure near normality of distribution for the transformed variables (Gill, 1978). Because the natural log of zero is indeterminant and the natural log of one is zero, one was added to each observation before the observation was con-

verted to a natural log value. Analysis of variance was performed using the General Linear Models procedure of the Statistical Analysis System maintained at Wayne State University. A procedure involving Bonferroni t statistics was utilized for comparisons among means (Gill, 1978). Because the gilts were assigned to dietary treatments, grouped by date farrowed into blocks and measured for trend in weight and serum parameters at several sampling times, a split-plot design was utilized. This design allows for the separation of random error into variation among and variation within subjects.

Results and Discussion

Split-plot design. Analysis of variance showed that sire did not affect the results. There was a significant interaction of treatment x block x time for weight and serum parameters, thus indicating nonparallel trends in response over time. Therefore, comparisons of treatments within blocks at each sampling time and comparison of means from each sampling time within treatment and block were made. Since the animals within each block were begun on the experiment at different times of year, their physiological state varied in the different seasons. As a result, block may interact with treatment and time.

Data which included the concentration of minerals (zinc, copper, iron) in tissues, sow productivity parameters, serum alkaline phosphatase activity and zinc concentration were logarithm transformed. A modified version of the Kolmogorov-Smirnov D-statistic was utilized to test for the probability of nonnormality. The residuals of the log-transformed data of number of pigs weaned, serum alkaline phosphatase activity and zinc concentration were considered to be normally distributed. The residuals of the other log-transformed variables were still

considered nonnormal but at a reduced probability. Numerous efforts did not reveal a more desirable transformation. The residuals of the variables body weight, serum copper concentration, glutamic-oxalacetic transaminase activity in serum, joint score and organ weight as a percent of body weight were nonnormal at a lesser probability than the residuals of the log transformed data and therefore, non-transformed data were analyzed. Log-transformed data are difficult to interpret even though this transformation improves the accuracy of the probability statement. Therefore, when the log-transformed means were utilized in the statistical analysis, the means of the original data are also provided in the tables for reference.

Effect of treatment within block and sampling period on body weight and age killed. Body weight was not significantly affected by treatment within block at 10, 14 and 18 months of age in blocks one and two (table 9). In block three, only two of the five gilts in the 5000 ppm treatment remained at 10 months of age. Their weight was significantly reduced at 14 and 18 months of age compared to the animals receiving no supplemental zinc in their diet. There was a trend for reduced weight for the highest supplemented group in all blocks. Weight and age killed are influenced by the overall health of the animal and the ability of the animal to conceive and produce viable offspring. The weight when killed was lower ($P<.05$) for animals receiving the 5000 ppm treatment in all blocks. Fifty-three percent of sows being fed 5000 ppm were removed from the study because of poor health before they had produced two litters. This compares to 7% for 500 ppm group, 20% for 50 ppm group and 14% for 0 ppm treatment group. In addition to these animals which had to be removed for health reasons,

Table 9. Sow weight (kg) and age data grouped by treatment and block

	Weight, kg											Age		Ratio	
	10 mo.			14 mo.			18 mo.			When killed		When killed, days		Killed wt./ age killed	
Treatment	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	Mean	SE	Mean	SE
Block 1																
0 ppm	5	165.9	7.3	5	194.6	7.3	3	203.8	9.4	5	187.2^{ab}	7.3	576.6	40.3	.334	.019
50 ppm	4	184.9	8.1	4	223.8	8.1	4	224.9	8.1	4	217.6^{a}	8.1	709.3	45.0	.307	.021
500 ppm	4	172.2	8.1	4	202.6	8.1	4	205.4	8.1	4	193.9^{ab}	8.1	669.5	45.0	.290	.021
5000 ppm	4	170.3	8.1	4	187.4	8.1	4	205.4	8.1	5	177.0^{b}	7.3	636.0	40.3	.279	.019
Block 2																
0 ppm	5	175.5	7.3	5	178.6	7.3	4	187.4	8.1	5	187.9^{a}	7.3	548.2	40.3	.347	.025
50 ppm	4	169.3	8.1	3	193.8	9.4	3	197.1	9.4	4	194.6^{a}	8.1	555.8	45.0	.357	.028
500 ppm	5	164.1	7.3	5	165.4	7.3	4	185.2	8.1	5	175.8^{a}	7.3	585.6	40.3	.299	.025
5000 ppm	4	137.2	8.1	3	163.6	9.4	2	160.0	11.5	5	131.4^{b}	7.3	434.2	40.3	.321	.025
Block 3																
0 ppm	5	141.4	7.3	5	186.0^{a}	7.3	4	193.3^{a}	8.1	5	205.4^{a}	7.3	558.6^{ab}	40.3	.369	.025
50 ppm	5	155.0	7.3	5	163.1^{ab}	7.3	5	181.4^{ab}	7.3	5	184.7^{a}	7.3	598.8^{a}	40.3	.309	.025
500 ppm	5	145.9	7.3	5	167.2^{ab}	7.3	5	189.5^{ab}	7.3	5	195.6^{a}	7.3	561.0^{ab}	40.3	.349	.025
5000 ppm	2	119.4	11.5	2	130.9^{b}	11.5	2	143.9^{b}	11.5	5	123.0^{b}	7.3	376.4^{b}	40.3	.354	.025
Overall[c] means	15	160.9		15	186.4		11	194.0		15	193.5		561.1			
	13	167.2		12	191.0		12	190.8		13	197.9		619.5			
	14	159.9		14	178.4		13	193.1		14	188.0		600.8			
	10	146.8		9	169.0		8	178.6		15	143.8		482.2			

[ab]Means in columns within blocks having different superscripts are significantly different ($P<.05$).

[c]For information only; not analyzed due to block effect.

two gilts failed to conceive from the 0 ppm treatment. Five animals from the group receiving 5000 ppm had severe rectal prolapses; four of these occurred at the first estrus after the boar had been put in the lot for field-mating. The fifth occurred a few days prior to parturition. Also, five sows in this treatment which produced one or more litters, were observed to have swollen vulvas prior to parturition which were twice to three times the size observed for sows on the other treatments. Estrogen in plasma rapidly declines immediately after estrus and remains low throughout the luteal phase until about day 16 when it rises rapidly to a peak at about day 18. Estrone and estradiol increase rapidly just prior to parturition and decline after parturition (Pond and Houpt, 1978). Most oral contraceptive agents (OCA) contain a combination of estrogens and progestogens and have been reported to produce increased copper levels in the plasma of users. Changes in the serum zinc with OCA use are not consistent. Crews et al. (1980) reported that the retention of these two nutrients is not affected by the use of oral contraceptives. Sows in this study receiving 5000 ppm zinc had significantly lower serum copper levels especially those that were observed with the rectal prolapse. The interrelationship between copper and estrogen which has not been elucidated may be involved in these observed abnormalities. The calculated ratio of weight when killed to age when killed (table 9) does not reflect gain per day because reproducing sows are gaining or losing weight depending on their stage of reproduction. However, it does follow the trend of weight when killed.

Effect of treatment within block on blood parameters. During the growth and development of these gilts (Hill et al., 1981), serum alkaline phosphatase (EC 3.1.3.1) activity was elevated in gilts receiving

5000 ppm zinc at most time periods. During their reproductive stages (table 10), this enzyme was again higher at 10 months of age in block 2. Alkaline phosphatase activity is usually elevated to 2 to 3 times normal in the sera of women in their third trimester of pregnancy (Fitzgerald et al., 1969). In this study, stages of gestation and lactation were not always similar within blocks. When comparing the activity of this enzyme in the sera of sows within treatments which were post-lactational and/or non-gravid with that of sows in various stages of gestation, this elevation during pregnancy does not occur (table 11). In fact, sows receiving 50 ppm had lower SAP during gestation than in the combined non-gravid and post-lactational stages, and sows receiving 5000 ppm had higher activity for this enzyme in the post lactation stage than in the other two stages. Also, the gilts which had been removed from the study due to rectal prolapse and several other animals suffered from severe lameness, which would probably affect the osteolytic activity of the animal. This will be discussed later. Values for the SAP activity of treatment groups within blocks (table 10) were not affected by time (10 vs 14 months). This would imply that the osteoblastic - osteolytic activity of the animals within a treatment remained constant. Because this enzyme requires zinc to function properly, decreased activity has been measured in baby pigs (Miller et al., 1968) when the animals were zinc deficient. Burch et al. (1975) did not observe a depression of this enzyme's activity in sera when pigs were fed the same amount of a zinc adequate (120 ppm) or zinc-deficient (7.3 ppm) diet. In this study, depressed activity of SAP was not observed in gilts/sows receiving the unsupplemented diet.

Table 10. Serum alkaline phosphatase activity (Sigma units/ml serum) by block and treatment

	Age, month						
	10			14			
		Mean			Mean		
Treatment	N	Mean	ln	SE	N	Mean	ln	SE
Block 1								
0 ppm	5	.588	.455[a]	.09	5	1.014	.688	.09
50 ppm	4	.818	.596[a]	.10	4	.980	.676	.10
500 ppm	5	1.098	.737[a]	.09	5	.722	.535	.09
5000 ppm	5	2.296	1.186[b]	.09	5	1.656	.958	.09
Block 2								
0 ppm	5	.808	.588	.09	5	.738	.542[a]	.09
50 ppm	4	.858	.609	.10	4	.925	.611[a]	.10
500 ppm	5	.744	.552	.09	5	1.202	.779[ab]	.09
5000 ppm	4	1.480	.854	.10	3	2.267	1.127[b]	.12
Block 3								
0 ppm	5	1.60	1.151[a]	.09	5	1.526	.917	.09
50 ppm	5	1.336	.825[ab]	.09	5	1.504	.912	.09
500 ppm	5	.760	.555[b]	.09	5	1.252	.802	.09
5000 ppm	2	5.140	1.815[c]	.14	2	2.415	1.214	.14

[abc]Means in columns within blocks having different superscripts are significantly different ($P<.05$).

Table 11. Effect of reproductive status on serum alkaline phosphatase activity and zinc and copper concentration

	Status								
	Non-gravid			Gravid			Post-lactation		
Treatment	N	Mean	SE	N	Mean	SE	N	Mean	SE
Serum alkaline phosphatase, Sigma units/ml									
0 ppm	6	1.15	.20	4	1.02	.24	5	1.08	.22
50 ppm[c]	3	1.42	.28	5	1.03	.22	2	1.63	.34
500 ppm	3	.95	.28	3	.83	.28	9	1.27	.16
5000 ppm	2	1.49[a]	.34	3	1.46[a]	.28	5	2.51[b]	.22
Serum zinc, µg/dl									
0 ppm	6	55	9.9	4	37	12.1	5	33	10.8
50 ppm[c]	3	69	14.0	5	38	10.8	2	66	17.1
500 ppm	3	56	14.0	3	42	14.0	9	62	8.1
5000 ppm	2	237[ab]	17.1	3	268[a]	14.0	5	217[b]	10.8
Serum copper, µg/dl									
0 ppm	6	196	20.5	4	184	25.1	5	169	22.4
50 ppm	3	246	28.9	5	206	22.4	2	226	35.4
500 ppm[c]	3	197	28.9	3	148	28.9	9	196	16.7
5000 ppm	2	78	35.4	3	76	28.9	5	91	22.4

[ab]Means on the same line with different superscripts differ significantly ($P<.05$).

[c]Non-gravid and post-lactation stages were significantly different ($P<.05$) compared to the gravid stages.

As observed in the growing and developing stages at all time periods (Hill et al., 1981), animals receiving 5000 ppm zinc in their diets had higher zinc concentrations in their serum at 10 and 14 months of age (table 12) than animals in all other treatments. Only in block two did the animals receiving 500 ppm have higher serum zinc values than those receiving 0 or 50 ppm. The concentration of zinc in the sera of many animals was below 50 μg/dl. This would often be considered typical of a deficient animal, but no other deficiency signs were observed. The reduced levels may be related to reproductive status. In comparing animals within treatment groups that are gravid with those which are not, only the serum zinc for sows receiving 50 ppm suppplemental zinc was lowered during gestation. Sows on the 5000 ppm added zinc treatment had elevated (table 11) concentrations of zinc when pregnant compared to the post-lactation stage. Johnson (1961) reported that serum zinc levels are decreased in pregnant women compared to their level at 8 weeks post-partum or to non-pregnant women. Bergmann et al. (1980) noted that hair zinc concentration tends to increase during pregnancy in mothers of infants with spina bifida and to decrease in the control group mothers. Schlicker and Cox (1968) reported death and variable degrees of resorption of the fetuses in rats fed 4000 ppm zinc. Ewes fed 700 ppm zinc have an increased incidence of perinatal death in their lambs (Underwood, 1977). Thus, the high zinc diet (5000 ppm) may be responsible for this group's reduced number of offspring and lower neonatal birth weight.

Serum copper concentrations (table 13) were significantly lower in the gilts/sows receiving 5000 ppm zinc at 10 and 14 months of age in all blocks than animals in all other treatments. Concentrations which

Table 12. Serum zinc (μg/dl) by treatment and block

	Age, months							
	10				14			
Treatment	N	Mean	Mean ln	SE	N	Mean	Mean ln	SE
Block 1								
0 ppm	5	58.50	4.016[a]	.12	5	44.60	3.810[a]	.12
50 ppm	4	59.25	4.086[a]	.14	4	38.75	3.680[a]	.14
500 ppm	5	83.20	4.425[a]	.12	5	41.20	3.722[a]	.12
5000 ppm	5	226.60	5.421[b]	.12	5	247.60	5.499[b]	.12
Block 2								
0 ppm	5	41.0	3.617[a]	.12	5	23.60	3.118[a]	.12
50 ppm	4	66.25	4.168[ab]	.14	4	29.75	3.328[a]	.14
500 ppm	5	72.40	4.272[b]	.12	5	51.60	3.953[b]	.12
5000 ppm	4	162.75	5.089[c]	.14	3	218.00	5.383[c]	.16
Block 3								
0 ppm	5	53.00	3.968[a]	.12	5	61.00	4.060[a]	.12
50 ppm	5	47.40	3.865[a]	.12	5	67.60	4.219[a]	.12
500 ppm	5	48.00	3.872[a]	.12	5	78.40	4.312[a]	.12
5000 ppm	2	198.50	5.273[b]	.19	2	235.00	5.459[b]	.19

[ab]Means in columns within blocks having different superscripts are significantly different ($P<.05$).

Table 13. Serum Copper (μg/dl) by treatment and block

	Age, months					
	10			14		
Treatment	N	Mean	SE	N	Mean	SE
Block 1						
0 ppm	5	179.4[a]	13.6	5	162.8[a]	13.6
50 ppm	4	169.5[ab]	15.2	4	209.0[a]	15.2
500 ppm	5	166.8[ab]	13.6	5	141.4[ab]	13.6
5000 ppm	5	110.4[b]	13.6	5	90.2[b]	13.6
Block 2						
0 ppm	5	183.0[ab]	13.6	5	153.4[a]	13.6
50 ppm	4	152.5[ab]	15.2	4	178.0[a]	15.2
500 ppm	5	202.6[a]	13.6	5	164.0[a]	13.6
5000 ppm	4	114.0[b]	15.2	3	71.0[b]	17.5
Block 3						
0 ppm	5	215.0[a]	13.6	5	235.4[a]	13.6
50 ppm	5	231.6[a]	13.6	5	206.8[a]	13.6
500 ppm	5	191.2[ab]	13.6	5	253.8[a]	13.6
5000 ppm	2	119.0[b]	21.5	2	88.0[b]	21.5

[ab]Means in columns within blocks having different superscripts are significantly different ($P<.05$).

were measured for this group (71 to 119 μg/dl) were much lower than what is accepted as normal levels (about 200 μg/dl). Elevated serum copper during pregnancy (Halsted et al., 1968; Pond and Houpt, 1978) has been reported for humans and swine, but this trend does not appear to occur within treatments (table 11) in this study. Sows receiving 500 ppm supplemental copper had lower serum copper concentrations during gestation than at the other two stages.

A copper deficiency results in low fertility and/or reproductive failure in many laboratory and farm animals (Underwood, 1977). It is not possible from this study to discern if the reduced productivity of the sows receiving 5000 ppm zinc is due to their reduced copper or elevated zinc status.

Serum glutamic-oxalacetic transaminase (SGOT) activity is a diagnostic tool used to aid in the identification of myocardial infarction, liver necrosis and lead and chemical poisoning. In humans, up to 28 Sigma-Frankel units/ml (SF) is considered normal, 28 to 50 SF is borderline and up to 2000 SF indicates liver necrosis. Piatkowski et al. (1979) reported values ranging from 14 to 52 SF for reproducing gilts. In this study, dietary treatments within blocks produced no difference in SGOT activity between groups at 10 and 14 months of age (table 14). From this one might conclude that SGOT is not a good measure of zinc toxicity or that zinc fed at 5000 ppm for 12 months does not produce measurable signs of liver necrosis or chemical poisoning in young gilts/sows.

Effect of treatment on sow productivity. There was not a significant block x treatment interaction so block was not considered in the data analysis. Pigs with incomplete skin covering, abnormal skeletal

Table 14. Glutamic-oxalacetic transaminase activity in serum of sows by treatment and block, Sigma-Frankel units/ml

	Age,months					
	10			14		
Treatment	N	Mean	SE	N	Mean	SE
Block 1						
0 ppm	5	28.6	5.5	5	11.2	1.4
50 ppm	4	35.2	6.2	4	13.7	1.5
500 ppm	5	26.0	5.5	5	9.8	1.4
5000 ppm	5	30.7	5.5	5	13.5	1.4
Block 2						
0 ppm	5	32.7	8.8	5	12.8	6.4
50 ppm	4	34.3	9.8	4	22.5	7.2
500 ppm	5	39.6	8.8	5	9.7	6.4
5000 ppm	4	46.0	9.8	3	12.7	8.9
Block 3						
0 ppm	5	32.3	3.0	5	12.1	3.9
50 ppm	5	43.6	3.0	5	10.7	3.9
500 ppm	5	36.7	3.0	5	11.2	3.9
5000 ppm	2	37.6	4.8	2	29.1	6.2

features, spraddled legs, a continual shaking condition and other gross external abnormalities were considered abnormal in this study. Sows on the unsupplemented diet (0 ppm added) had a significantly higher number of abnormal pigs per litter while sows receiving 500 ppm had the lowest number of abnormalities per litter (table 15). Wegger and Palludan (1977) found that a zinc deficiency during different periods of gestation did not affect litter size or birth weight but reduced viability of the offspring, and abnormalities were rather insignificant. This is in contrast with the increased number of abnormalities and reduced litter size and weight observed by Hurley and Swenerton (1966) with rats.

There were fewer pigs weaned by sows on the highest supplementation level than by sows receiving 50 to 500 ppm zinc in their diet. The number of pigs weaned per litter in all treatments is lower than what might normally be observed in the swine industry because two pigs were killed at birth from each litter. The weaning weight of pigs from sows fed 5000 ppm was less than for pigs whose dams received 0 or 500 ppm. If the viability of the pigs from dams receiving no supplemental zinc had been decreased, the number of pigs weaned and the weaning weight should have been reduced. This did not occur. There was a trend for sows receiving the 500 ppm zinc supplement to produce and wean heavier pigs than sows on the other treatments.

Effect of treatment on tissues and their mineral concentrations. Because animals were killed at different weights, it was necessary to express organ weight as a percent of body weight (% BW) to determine if treatment had affected organ size. Liver weight as % BW was increased in sows fed 5000 ppm zinc compared to liver weights from sows fed no

Table 15. Effect of treatment on sow productivity

Treatment	N	Mean total pigs	Mean ln total pigs	SE	Mean total live pigs	Mean ln total live pigs	SE
0 ppm	20	9.84	2.35	.12	9.30	2.30	.13
50 ppm	23	9.57	2.29	.11	8.91	2.23	.12
500 ppm	23	10.17	2.38	.11	9.57	2.33	.12
5000 ppm	15	9.07	2.21	.14	8.40	2.14	.15

	Mean average birth wt.(g)	Mean ln avg. birth wt.	SE	Mean no. abnormal/ litter	Mean ln no. abnormal/litter	SE
0 ppm	1498.6	7.30	.38	1.05	.34[a]	.06
50 ppm	1457.7	7.28	.35	.30	.20[b]	.06
500 ppm	1394.1	7.22	.35	.13	.08[c]	.06
5000 ppm	1254.8	7.11	.44	.33	.19[b]	.07

	Mean pigs weaned	Mean ln pigs weaned	SE	Mean Avg. weaning wt.(g)	Mean ln avg. weaning, wt.	SE
0 ppm	6.00	1.80[ab]	.15	4987.6	8.16[a]	.47
50 ppm	6.13	1.82[a]	.14	4970.8	7.83[ab]	.44
500 ppm	6.43	1.89[a]	.14	4993.2	8.17[a]	.44
5000 ppm	4.82	1.33[b]	.17	3603.3	6.66[b]	.55

[ab]Means in columns within variables having different superscripts are significantly different ($P<.05$).

Table 16. Effect of treatment on organ weight as a percent of body weight of sows

		Organ									
Treatment	N	Heart	SE	Liver	SE	Spleen	SE	Pancreas	SE	Kidney	SE
0 ppm	15	.295	.02	1.09[a]	.11	.169[ab]	.01	.119	.01	.227	.02
50 ppm	11	.318	.02	1.20[ab]	.12	.175[ab]	.01	.118	.01	.244	.03
500 ppm	13	.336	.02	1.23[ab]	.11	.179[a]	.01	.138	.01	.241	.03
5000 ppm	15	.315	.02	1.46[b]	.11	.142[b]	.01	.104	.01	.281	.02

[ab]Means in columns with different superscripts are significantly different ($P<.05$).

supplemental zinc (table 16). Miller et al. (1968) did not observe an effect on organ size in zinc-deficient baby pigs in any of the organs weighed in this study. Spleen weight as % BW was decreased for sows fed 5000 ppm zinc compared to those fed 500 ppm. This does not follow the expected direction if these sows were marginally copper-deficient. Copper-deficient rats have been observed to have cardiac hypertrophy and enlarged spleens (Underwood, 1977). Copper-deficient pigs have also been observed to have cardiac hypertrophy. Heart size was not significantly affected by treatment in this study.

The concentration of minerals in tissues is expressed on a wet weight basis because the percent dry matter was not significantly different between treatments. The overall mean for percent dry matter for each organ was as follows: liver, 29.3%; kidney, 20.9%; spleen, 21.8%; muscle, 25.9%; heart, 22.2%; pancreas, 29.6% and aorta, 32.6%.

The concentration of zinc in the liver increased significantly with the progressively higher dietary zinc treatments (table 17). Cox and Hale (1962) observed similar additive zinc stores in the liver of pigs when a basal, +2000 ppm and +4000 ppm zinc diets were fed for 69 days. Hamilton et al. (1979) found increasing zinc depositions in the liver of Japanese quail when the diet provided progressively higher levels of zinc (0 to 2000 ppm) with 1 ppm or 3.6 ppm copper in the diet. The level of copper in the diet did not affect the level of zinc in the liver. Ott et al. (1966a) reported that the zinc concentration in lamb's liver increased with increasing levels of dietary zinc to 2000 ppm; but 4000 ppm in the diet did not produce a further increase. This same group (Ott et al., 1966b) reported that up to 1700 ppm of zinc added to the diet of calves increased liver stores, but 2100 ppm

Table 17. Effect of treatment on concentration of zinc, copper and iron in tissues of sows (ppm, wet basis)

		Zn			Cu			Fe			
			Mean			Mean			Mean		%
Treatment	N	Mean	In	SE	Mean	In	SE	Mean	In	SE	DM
						Liver					
0 ppm	15	55.67	3.90[a]	.08	17.06	2.67[a]	.06	238.2	5.35[ab]	.11	30.6
50 ppm	13	63.62	4.14[b]	.08	10.08	2.34[b]	.07	271.0	5.58[ab]	.12	28.9
500 ppm	14	90.07	4.46[c]	.08	7.76	2.12[b]	.07	322.9	5.74[a]	.12	30.4
5000 ppm	14	1037.43	6.91[d]	.08	2.59	1.26[c]	.07	221.4	5.32[b]	.12	27.4
Treatment						Heart					
0 ppm	15	17.00	2.89	.08	3.83	1.57	.06	51.27	3.95	.11	21.9
50 ppm	13	17.62	2.92	.08	3.96	1.60	.07	46.08	3.84	.12	21.9
500 ppm	14	15.00	2.77	.08	3.98	1.60	.07	44.14	3.80	.12	22.4
5000 ppm	10	18.30	2.95	.09	3.04	1.39	.08	41.55	3.49	.14	22.5
Treatment						Kidney					
0 ppm	15	29.33	3.29[a]	.08	8.34	2.15[a]	.06	57.00	4.04	.11	20.4
50 ppm	13	23.15	3.17[a]	.08	7.21	2.08[a]	.07	66.92	4.19	.12	21.1
500 ppm	14	29.50	3.40[a]	.08	8.67	2.22[a]	.07	61.21	4.10	.12	21.7
5000 ppm	15	366.73	5.53[b]	.08	22.17	3.05[b]	.06	64.00	4.04	.11	02.5
Treatment						Pancreas					
0 ppm	14	32.14	3.48[a]	.08	1.54	.93	.07	19.79	3.02	.12	30.2
50 ppm	13	38.00	3.62[a]	.08	1.35	.85	.07	18.77	2.97	.12	29.7
500 ppm	14	39.71	3.68[a]	.08	1.26	.82	.07	20.14	3.03	.12	27.7
5000 ppm	10	974.70	6.60[b]	.09	1.35	.84	.08	22.42	2.74	.14	31.1
Treatment						Spleen					
0 ppm	15	23.13	3.18	.08	1.01	.69	.06	743.1	6.46	.11	21.8
50 ppm	13	20.62	3.07	.08	.95	.67	.07	757.4	6.41	.12	21.6
500 ppm	14	21.07	3.09	.08	.99	.67	.07	711.2	6.43	.12	21.8
5000 ppm	15	24.60	3.24	.08	.81	.59	.06	574.7	6.24	.11	22.0
Treatment						Muscle					
0 ppm	15	21.80	3.06	.08	.56	.44	.06	11.28	2.48	.11	25.8
50 ppm	13	22.92	3.13	.08	.65	.50	.07	11.93	2.55	.12	25.4
500 ppm	14	24.07	3.19	.08	.72	.54	.07	14.27	2.70	.12	26.3
5000 ppm	15	20.60	3.02	.08	.57	.45	.06	12.44	2.58	.11	25.8
Treatment						Aorta					
0 ppm	15	18.33	2.95[a]	.08	.83	.60[a]	.06				32.8
50 ppm	13	17.69	2.91[a]	.08	.83	.60[a]	.07				32.3
500 ppm	14	21.50	3.10[ab]	.08	.77	.57[ab]	.07				36.1
5000 ppm	9	26.44	3.31[b]	.10	.53	.42[b]	.08				28.3

[ab]Means in columns within tissues with different superscripts are significantly different ($P<.05$).

did not appear to produce further deposition of this element. Unlike other species, supplementation of the chick with 600 or 1200 ppm added zinc did not elevate tissue (kidney and liver) zinc levels. At 2400 ppm of added dietary zinc, liver zinc concentrations were increased (Kincaid et al., 1976). Oh et al. (1979) found that the highest percent of the total hepatic zinc was in the soluble fraction of the liver when either 1000, 2000, 4000 or 8000 ppm of zinc was added to a chick's diet. When 16,000 ppm were fed, a higher portion of the zinc deposited in the liver was in the crude nuclei and mitochondrial fraction. Thus, subcellur distribution appears affected at this higher dietary level. In our study, zinc was not increased significantly in the kidney except with the 5000 ppm dietary zinc treatment. Similar results were observed with the chick when 2400 ppm zinc was fed by Kincaid et al. (1976). Oh et al. (1979) noted that the greatest percent of renal zinc was in the soluble fraction regardless of dietary zinc level. It appears that homeostatic control mechanism(s) of these two organs may be affected by different levels of dietary zinc.

The amount of zinc in the pancreas of animals receiving 0, 50 or 500 ppm supplemental dietary zinc was within the expected range (Underwood, 1977), but was only about 4% of the amount found in the pancreas of gilts/sows fed 5000 ppm added zinc. It appears that the pancreas, like the kidney, has an effective homeostatic mechanism in the pig at these lower levels of supplementation. However, Oh et al. (1979) found the highest percent of pancreatic zinc in the soluble fraction when 1000, 2000 or 4000 ppm of dietary zinc were added to the diet of chicks, but most of the pancreatic zinc was in the crude nuclei and mitochondrial fraction when 8000 or 16000 ppm were added.

There was a significantly higher amount of zinc in the aorta of gilts/sows fed the highest level of dietary supplementation compared to 0 or 50 ppm. Because of the importance of a copper requiring enzyme, lysyl oxidase, for collagen cross-linking the the aorta, it was hypothesized that high dietary zinc might reduce the copper available in this tissue. Copper in the aorta was reduced in gilts/sows on the highest zinc treatment compared to those receiving 0 or 50 ppm additonal zinc. Therefore, zinc and copper exhibit the classic inverse relationship of high zinc and low copper.

This inverse relationship can also be observed in the zinc and copper concentrations in the liver (table 17). Cox and Hale (1962) did not find hepatic copper reduced when zinc was added at 2000 or 4000 ppm compared to a basal diet. Ott et al. (1966b) found similar results with calves fed up to 1700 ppm, but like the zinc concentration for animals fed 2100 ppm, copper also increased. Lambs (Ott et al., 1966a) showed decreasing copper with increasing levels of dietary zinc except there was no difference in the mean hepatic copper stores between lambs fed either 2000 or 4000 ppm dietary zinc. In this study, the kidney was the only tissue where higher levels of copper were found in animals receiving the 5000 ppm dietary zinc treatment compared to the other treatments (table 17). Cox and Harris (1960) did not observe an effect on renal copper in rats fed 5000 or 6000 ppm zinc. Utilizing radioactive ^{64}Cu, Magee and Matrone (1960) reported that 13.4% of the absorbed copper was present in the kidney tissue and 45.5% in the urine of rats fed 7500 ppm supplemental zinc compared to 17.8% in the kidneys and 21.7% in the urine for the controls. The radioactive dose was given via stomach tube and the values were reported as a percent of

absorbed copper so it is not possible to compare the absolute amount of copper in the kidneys. Van Campen (1966) reported that a reduced percent of radioactive ^{64}Cu dose was recovered in tissues if the Zn/Cu ratio was 600 compared to 0, but that the percent of the absorbed dose was the same in the kidney tissue. Copper-deficient pigs have reduced renal copper stores. Hypothetically, the gilts/sows on the high zinc treatment could be attempting to retain copper to compensate for the reduced copper levels in other tissue, or the urine could be an excretory route for copper when zinc is fed at excessive levels in the diet. Stores of copper and iron in the heart, pancreas, spleen and muscle were not affected by dietary treatment.

Hepatic iron stores were lower in gilts/sows fed 5000 ppm zinc compared to those fed 500 ppm. Cox and Harris (1960) and Magee and Matrone (1960) with rats, Cox and Hale (1962) with pigs and Hamilton et al. (1979) with Japanese quail have reported similar results. It is not clear why there is not a difference in hepatic iron stores when the high zinc group is compared to the 0 or 50 ppm supplemented groups.

Effect of dietary treatments on necropsy observations. Scores on both the left and right humeral-radial/ulnar joints were significantly higher for gilts/sows receiving 5000 ppm zinc than those observed for the animals on the other treatments (table 18). Lameness had been observed in many of the animals prior to death. Sampson et al. (1942) reported stiffness, lameness and the abnormal development of leg bones in young pigs fed .1% zinc from zinc lactate in milk. They reported the joint capsules of the shoulder, elbow, hip and stifle joints to be distended abnormally and to contain thick, blood-tinged synovial fluid as well as other gross pathologic changes which they referred to as

Table 18. Effect of treatment on the humeral-radial/ulnar joint of sows when scored for osteochondrosis (0 = no effect, 5 = severe)

Treatment	N	Left	SE	Right	SE
0 ppm	15	.87[a]	.28	.67[a]	.31
50 ppm	13	1.81[a]	.30	1.65[a]	.33
500 ppm	14	1.11[a]	.29	1.32[a]	.32
5000 ppm	15	3.80[b]	.28	2.90[b]	.31

[ab]Means in columns with different superscripts are significantly different ($P<.05$).

arthritis. Brink et al. (1959) reported that young pigs fed 2000, 4000 or 8000 ppm of zinc from zinc carbonate exhibited toxic signs which included reduced gain, feed intake, feed efficiency; arthritis, extensive hemorrhage in axillary spaces, gastritis, catarrhal enteritis, congestion of the mesentery, and hemorrhages in the ventricles of the brain, lymph nodes and spleen. Hemoglobin values determined after 21 days on the diets were not affected by dietary treatment.

In this study, the proximal extremity of the humerus and other long bones (which were not scored) often exhibited abnormal articular cartilage. Some areas on the condyle of the bone were eroded and therefore were very thin with little or no cartilage present. Excessive synovial fluid was found in the joints of some animals. Often the articulating cartilaginous surface contained numerous fracture or suture lines and/or abnormal proliferation. Four sows receiving 50 ppm supplemental zinc and one sow receiving 500 ppm had mild (score of less than 2) joint lesions which appeared to be healing.

In 1970 Siegle and Martin (1970a) purified the enzyme, lysyl oxidase, from cartilage and from bone (Siegel et al., 1970b). Since that time, Harris and O'Dell (1974) have established that copper is specific for lysyl oxidase function. Because of the reduced copper present in the body of animals receiving 5000 ppm supplemental zinc, it is hypothesized that the observed joint abnormalities may result from the reduced activity of lysyl oxidase. Neither bone nor aortic tissue were assessed for the activity of this enzyme in this study.

Other gross pathological observations of gilts/sows which were fed this high level of dietary zinc include: gastritis; enteritis; severe neurological disturbance which prevented a normal stance and was

characterized by a hyper-responsive reaction to touch; hemorrhaging in axillary space and abdominal cavity, necrosis of kidney; fibrinous tags along corda tendona; excessive fluid in thoracic and abdominal cavities; dilation of vena cava; hyperkeratinization in stomach and small intestine and white muscle. One sow receiving 500 ppm supplemental zinc was killed after her first parity because she was lame, had difficulty in getting up and had abcesses on both sides of the tail. Her joints received 1 ratings and gross observation did not reveal reasons for her condition. One sow with no added zinc had white muscle disease and another was killed due to her refusal to eat. Necropsy revealed that one fetus was present and that the placenta was not viable. Two sows receiving 50 ppm supplemental zinc had pericardial adhesions. Detailed histology of tissues from these animals will be published at a later date.

Data from this study would indicate that even though sows fed 5000 ppm zinc were able to produce viable offspring, their health and productivity were reduced. In general, sows receiving 50 or 500 ppm supplemental zinc had very similar production, blood and health parameters, but there was a trend for sows on the 500 ppm diet to have fewer health problems while producing more pigs which were heavier at weaning.

SECTION III

Concentration of Minerals in Tissues of Pigs From Dams Fed Different Levels of Dietary Zinc

Introduction

There are many known interactions among elements that have captured the attention of researchers. Because of the physicochemical properties of ions with similar valence shell electronic structures, many elements are believed to be biologically antagonistic. Chemical combinations of ions which do not have similar electronic configurations may also result in antagonistic interactions. The mineral components of our environment are continually altered by the pollution of natural resources, but the long term effects on man, plants and animals and their progeny are not predictable. More information is needed to uncover unknown interrelationships, to discover the underlying mechanisms and to predict the consequences of environmental aberrations. Schlicker and Cox (1968) observed the effects of high levels of zinc on adult female rats and their offspring, and James et al. (1966) studied the effects of sublethal doses of certain minerals including zinc on pregnant ewes and their fetuses. Several workers have studied the teratological effects on offspring of dams fed a zinc-deficient diet. No data are available on the effects of various levels of zinc in the maternal diet of the pig on the relative organ size and concentration of calcium, phosphorus, manganese, iron, copper and zinc in several tissues of the progeny. Thus, the purpose of this investigation was to determine, report and discuss this type of data.

Experimental Procedure

Experimental animals. The dams of the pigs used in this study were fed a corn-soybean meal basal diet supplemented to meet known dietary requirements except zinc from 30 kg until completion of the study. The four treatments were the addition of 0, 50, 500 or 5000 ppm of zinc from zinc oxide to the basal diet which contained from 35 to 38 ppm zinc. Details about how these gilts/sows were handled and information about dietary content are reported in previous papers (Hill et al., 1981a,b).

Weighing, blood and tissue sampling and analyses. Within 24 hours after birth and at 21 days of age, all live pigs were weighed and one male and one female were killed from each litter. When only one sex was represented in the live pigs, one pig was killed. Blood samples were obtained from all second parity pigs from the anterior vena cava and partitioned into two components for determining (1) serum copper and zinc and (2) hemoglobin and hematocrit concentrations. Pigs were killed by injection of a saturated magnesium sulfate solution into the anterior vena cava to render them insensible followed by exsanguination. Organs were removed, weighed, placed in plastic bags and frozen at -20°C until analyzed. The liver, heart, kidney, pancreas, adrenal glands, thyroid gland, spleen and esophagus were obtained from all pigs in both parities. The aorta was removed from pigs whose mothers were fed 50 or 5000 ppm zinc in the first parity and from all pigs in the second parity. Testes were removed from all males in the second parity.

Serum was diluted 1:7 with deionized-distilled water for determination of copper and zinc by atomic absorption spectrophotometry (IL-453, Instrumentation Laboratory, Lexington, MA). Hemoglobin con-

centrations were determined by the cyanmethemoglobin method of Crosby et al. (1954) and hematocrits by the procedure of McGovern et al. (1955). Uniform samples were cut from tissues, and duplicate samples were wet digested in a mixture of nitric and perchloric acids and diluted with deionized-distilled water as necessary for analyses. Copper, iron, zinc, calcium and manganese determinations were made by atomic absorption spectrophotometry (IL-453, Instrumentation Laboratory, Lexington, MA). For calcium analyses, strontium chloride was used to reduce matrix interference. Phosphorus determinations were made by use of the Gomori modification of the Fiske and Subbarow procedure (Gomori, 1942). Only tissues from first parity pigs were analyzed for calcium, manganese and phosphorus.

Statistical analyses. A modified version of Kolmogorov-Smirnov D-statistic was used to test for the probability of non-normality of distribution. Because the data were distinctly nonnormal, a natural logarithm transformation was utilized to ensure near normality of distribution for the transformed variables (Gill, 1978). Because the natural log of zero is inderterminant and the natural log of one is zero, one was added to each observation before the observation was converted to a natural log value. Analysis of variance was performed using the General Linear Models procedure of the Statistical Analysis System maintained at Wayne State University. A procedure involving Bonferroni t statistics was utilized for comparisons among means (Gill, 1978). A split-plot design was utilized because the pigs were assigned to the dietary treatment of their dam, killed at 1 or 21 days of age and were of either sex. Dams were blocked by the date they were farrowed.

Results and Discussion

Split-plot design. Analysis of variance showed that the dam's sire and block and the sex of the pig did not affect the results. There was a significant tissue x treatment x parity interaction for the concentration of iron, zinc and copper in organs analyzed. Therefore, comparisons within parities and tissue of treatments and comparisons of the effect of parity within tissue and treatment were made. A significant interaction exists for tissue x treatment for the concentration of calcium, phosphorus and magnesium in the organs analyzed. Age significantly affected the concentration of the zinc in the liver and serum, copper in the serum, phosphorus in the liver and the testes weight expressed as a percent of body weight. Only the effect of age on these variables will be presented.

After the natural logarithm transformations of the data were completed, a modified version of the Kolmogorov-Smirnov D-statistic was utilized to test for the probability of nonnormality. The residuals of the kidney and heart weight as a percent of body weight were considered to be normally distributed. The residuals of the pancreas, adrenal glands and thyroid gland weight expressed as a percent of body weight, and the concentration of copper and zinc in tissues natural log-transformed variables were still considered nonnormal but at a reduced probability. A more desirable transformation was not found. The residuals of the variables of testes, liver, heart and spleen weight as a percent of body weight and concentration of iron, calcium, phosphorus and magnesium in tissues were nonnormal at a lesser probability than the residuals of the natural log transformed data. Therefore, non-transformed data were analyzed. The means of the original data are provided in the

tables for reference since log-transformed data are difficult to interpret even though it improves the accuracy of the probability statements.

Effect of dam's treatment and parity on organ weight. Because pigs were killed at different weights, it was necessary to express organ weight as a percent of body weight (relative organ weight) to determine if treatment or parity within treatment had affected organ size. Pigs from second parity sows that were fed a dietary addition of 5000 ppm zinc had significantly lower relative kidney weights than did pigs from the first parity (table 19). Miller et al. (1967) noted that six week old pigs which were fed diets deficient in iron, calcium, phosphorus, vitamin D or magnesium had significantly lower relative kidney weights than did control animals that were supplemented with the missing nutrient. Pigs from dam's fed the highest addition of zinc (5000 ppm) had significantly larger relative adrenal and thyroid glands compared to pigs from dam's fed 50 or 500 ppm additional zinc. Relative adrenal gland weights were increased in iron, calcium, phosphorus, vitamin D or magnesium-deficient pigs and the relative thyroid weight from pigs deficient in calcium and magnesium were increased compared to controls in the work reported by Miller et al. (1967). More recently, Morley et al. (1980) reported that zinc-deficient rats had increased thryoid weights per 100 g body weight and lower triiodothyronine and thyroxine levels compared to ad libitum controls. It would appear that the hypertrophy of this organ is a response to the decreased secretory ability of the organ. "Stress" can be any kind of a stimulus that causes the hypothalamus to release corticotropin-releasing hormone (CRH). An increased release of adrenocorticotrophic hormone corticotropin (ACTH) results from this higher level of CRH. Ultimately, the

Table 19. Effect of dam's treatment and parity on organ weight as a percent of body weight

Dam's treatment and tissue	1 Parity N	Mean	Mean ln	SE	2 Parity N	Mean	Mean ln	SE	Combined parities N	Mean	Mean ln	SE
Kidney												
0 ppm	47	.702	.528	.011	31	.634	.488	.014	78	.675	.512	.011
50 ppm	50	.705	.531	.011	39	.657	.502	.012	89	.684	.519	.010
500 ppm	50	.695	.525	.011	39	.634	.488	.012	89	.668	.509	.010
5000 ppm	26	.765	.566[a]	.015	21	.630	.481[b]	.017	47	.705	.528	.014
Pancreas												
0 ppm	47	.133	.124	.004	31	.125	.117	.005	78	.130	.121	.004
50 ppm	50	.130	.121	.004	39	.126	.118	.005	89	.128	.120	.004
500 ppm	50	.126	.118	.004	39	.114	.107	.005	89	.121	.113	.004
5000 ppm	26	.130	.122	.006	21	.116	.109	.006	47	.124	.113	.005
Adrenal glands												
0 ppm	47	.020	.019	.0010	31	.019	.019	.0012	78	.019	.019[cd]	.0009
50 ppm	50	.017	.017	.0009	39	.018	.017	.0011	89	.017	.017[c]	.0009
500 ppm	50	.018	.017	.0009	39	.018	.018	.0011	89	.018	.018[c]	.0009
5000 ppm	26	.021	.021	.0013	21	.023	.023	.0014	47	.022	.022[d]	.0012
Thyroid gland												
0 ppm	47	.017	.017	.0009	31	.019	.019	.0012	78	.018	.018[cd]	.0007
50 ppm	50	.015	.015	.0009	39	.018	.018	.0010	89	.017	.016[c]	.0007
500 ppm	50	.015	.015	.0009	39	.019	.018	.0010	89	.017	.016[c]	.0007
5000 ppm	26	.018	.018	.0012	21	.021	.021	.0014	47	.020	.019[d]	.0009
Liver												
0 ppm	47	2.43		.06	31	2.28		.07	78	2.37[c]		.05
50 ppm	50	2.52		.07	39	2.41		.06	89	2.47[c]		.04
500 ppm	50	2.54		.07	39	2.32		.06	89	2.44[c]		.04
5000 ppm	26	2.77		.09	21	2.79		.09	47	2.78[d]		.06
Heart												
0 ppm	47	.55		.01	31	.57		.02	78	.56[c]		.01
50 ppm	50	.56		.01	39	.58		.01	89	.57[c]		.01
500 ppm	50	.54		.01	39	.56		.01	89	.55[c]		.01
5000 ppm	26	.63		.02	21	.67		.02	47	.65[d]		.01
Spleen												
0 ppm	47	.16		.01	31	.15		.01	78	.15		.01
50 ppm	50	.14		.01	39	.13		.01	89	.14		.01
500 ppm	50	.14		.01	39	.15		.01	89	.14		.01
5000 ppm	26	.15		.01	21	.11		.02	47	.13		.01

[ab]Values for parities within treatment and tissue (same line) which have different superscripts differ significantly ($P<.05$).

[cd]Values for treatments (combined parities) within tissues which have different superscripts in columns within tissue differs significantly ($P<.05$).

ACTH stimulates the adrenal cortex to secrete corticosteroids. ACTH can stimulate an increase in size and weight of the adrenal glands. In humans, estrogens increase the sensitivity of the pituitary ACTH releasing process to CRH from the hypothalmus, thereby affecting the adrenal glands and their secretions (Yates et al., 1974). As reported previously (Hill et al., 1981a,b), there appeared to be an interaction between the reduced copper seen in the sows fed 5000 ppm zinc and the estrogens involved in the sow's reproductive cycle. Thyroid hormones increase the rate of corticosteroid destruction by the liver which would reduce circulating cortisol levels and stimulate the release of ACTH. Therefore, this additional ACTH secretion rate could cause adrenal hypertrophy (Yates et al., 1974). Pigs from sows fed 5000 ppm additional zinc had heavier relative liver and heart weights than did pigs from sows on the other treatments. The sow's nutrition during gestation appears to be an important factor. The dam's treatment did not affect the relative testes weight, but intact males which were 21 days of age had higher relative testes weight than did intact males at 1 day of age regardless of treatment (table 20). This tissue was only obtained from second parity pigs.

Effect of dam's treatment and parity on the concentration of minerals in tissues. The mean percent dry matter of the liver, heart, kidney and pancreas are given in table 21. The mean percent dry matter in the esophagus were 19.6, 20.2, 19.6 and 22.5 and in the testes were 22.0, 20.7, 20.1, and 19.9 respectively, for the dam's treatments of 0, 50, 500 and 5000 ppm added zinc.

Table 20. Effect of dam's treatment and age on testes weight as a percent of body weight (2nd parity)

Dam's	Age					
	1 day			21 days		
treatment	N	Mean	SE	N	Mean	SE
0 ppm	9	.044[a]	.008	7	.134[b]	.009
50 ppm	7	.046[a]	.009	9	.115[b]	.008
500 ppm	10	.050[a]	.008	9	.096[b]	.008
5000 ppm	7	.056[a]	.009	3	.151[b]	.014

[ab]Values on the same line with different superscripts differ significantly ($P<.05$).

Table 21. Effect of treatment and parity on the concentration of iron in tissues of offspring (ppm, wet basis)

Dam's treatment and tissue	Parity 1 N	Mean	SE	% Dry matter	Parity 2 N	Mean	SE	% Dry matter
Liver								
0 ppm	43	232.2[ac]	18.6	26.9	31	159.1[bc]	21.9	26.3
50 ppm	50	291.6[ac]	17.3	27.1	39	203.0[bc]	19.6	26.3
500 ppm	49	274.6[ac]	17.4	26.9	39	213.9[bc]	19.6	26.5
5000 ppm	26	387.7[d]	24.0	26.8	21	352.3[d]	26.7	26.8
Heart								
0 ppm	43	40.12	18.6	20.5	31	32.65	21.9	20.1
50 ppm	49	40.76	17.4	20.9	39	32.44	19.6	20.2
500 ppm	50	39.48	17.3	20.0	39	35.33	19.6	19.9
5000 ppm	26	39.42	24.0	20.1	21	39.43	26.7	20.2
Kidney								
0 ppm	43	52.79	18.6	19.3	31	40.61	21.9	18.9
50 ppm	50	61.04	17.3	19.1	38	41.61	19.8	19.3
500 ppm	50	59.00	17.3	19.3	39	43.74	19.6	18.8
5000 ppm	26	53.38	24.0	18.8	21	39.67	26.7	19.5
Pancreas								
0 ppm	43	37.79	18.6	24.3	29	27.28	22.7	24.7
50 ppm	49	37.76	17.4	23.6	39	32.85	19.6	24.5
500 ppm	48	33.83	17.6	24.0	39	30.38	19.6	23.4
5000 ppm	26	43.69	24.0	23.5	21	28.19	26.7	24.0

[ab]Values for parities within treatment and tissue (same line) which have different superscripts differ significantly ($P<.05$).

[cd]Values for treatments which have different superscripts in the columns within tissues differ significantly ($P<.05$).

Iron was significantly increased in the liver of first and second parity pigs from sows fed 5000 ppm zinc compared to the other treatments (table 21).

Schlicker and Cox (1968) reported that the concentration of whole body iron in 16 and 18 day old rat fetuses whose dams had been fed 4000 ppm zinc was lower than the concentration in control fetuses. However, hepatic iron stores from 18 and 20 day old fetuses of dams on the high zinc diet were not different statistically from that of the controls but tended to be higher. Cox and Harris (1960) reported that iron stores in the liver of rats fed 4000 or 5000 ppm zinc were reduced. Similar results in rats were reported by Magee and Matrone (1960) and Scott and Magee (1979). Cox and Hale (1962) noted that pigs fed 4000 ppm zinc for 69 or 96 days had lower hepatic iron stores than pigs fed 2000 ppm or 40 ppm zinc. Ritchie et al. (1963) reported that 100 ppm added zinc fed for 15 weeks resulted in higher hepatic iron stores than those found in pigs fed the following additions to a basal diet: 100 ppm zinc and 250 ppm copper, 250 ppm copper, 125 ppm copper or 1.3% calcium. The iron concentration in the liver of the second parity pigs was lower than that of first parity pigs within treatment. This parity affect on mineral transfer does not appear to have been studied as much as the affect of stage of gestation and physiological age and size of the fetus. The iron content of the heart, kidney and pancreas was not affected by dietary treatment and parity.

The zinc concentration in the liver was increased in the 21 day old pigs from both parities compared to the 1 day old when their dams received 0, 50 or 500 ppm supplemental zinc in their diet (table 22). However, pigs from sows that received 5000 ppm additional zinc in their

Table 22. Effect of age on concentration of zinc in the liver (ppm, wet basis) of pigs from sows fed 0, 50, 500 or 5000 ppm supplemental zinc

	Parity 1						Parity 2					
Dam's	1 Day			21 Day			1 Day			21 Day		
treatment	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
0 ppm	24	52[ac]	23	23	74[bc]	24	16	44[ac]	28	15	105[bc]	29
50 ppm	25	82[ac]	23	25	115[bc]	23	20	66[ac]	25	19	103[bc]	26
500 ppm	24	88[ac]	23	24	129[bc]	23	21	71[ac]	25	19	121[bc]	26
5000 ppm	15	1310[ad]	29	11	514[bd]	34	14	1200[ad]	30	7	792[bd]	43

[ab]Means on the same line with the same parity with different superscripts differ significantly ($P<.05$).

[cd]Means in the same column with different superscripts differ significantly ($P<.05$).

Table 23. Effect of treatment and parity on the concentration of zinc in tissues of offspring (ppm, wet basis)

Dam's treatment and tissue	Parity 1 N	Mean	Mean ln	SE	Parity 2 N	Mean	Mean ln	SE
Liver								
0 ppm	43	61.3	3.91[ac]	.05	31	73.5	4.15[bc]	.06
50 ppm	50	100.4	4.46[d]	.05	39	84.0	4.32[cd]	.06
500 ppm	50	107.4	4.44[d]	.05	39	96.4	4.39[d]	.06
5000 ppm	26	974.3	6.71[e]	.07	21	1064.7	6.92[e]	.08
Heart								
0 ppm	43	17.7	2.90	.05	31	15.4	2.79	.06
50 ppm	49	15.9	2.82	.05	39	16.3	2.84	.06
500 ppm	50	16.6	2.87	.05	39	15.6	2.80	.06
5000 ppm	26	16.2	2.84	.07	21	17.9	2.93	.08
Kidney								
0 ppm	43	16.1	2.83[c]	.05	31	17.5	2.91[c]	.06
50 ppm	50	15.4	2.79[c]	.05	38	16.6	2.86[c]	.06
500 ppm	50	16.5	2.85[c]	.05	39	16.5	2.84[c]	.06
5000 ppm	26	25.4	3.23[d]	.07	21	28.0	3.20[d]	.08
Pancreas								
0 ppm	43	29.4	3.35[c]	.05	29	29.4	3.29[c]	.07
50 ppm	49	33.1	3.44[acd]	.05	39	25.7	3.15[bc]	.06
500 ppm	49	36.6	3.53[d]	.05	39	39.6	3.60[d]	.06
5000 ppm	26	238.7	5.41[e]	.07	21	247.0	5.41[e]	.08
Esophagus								
0 ppm	42	14.1	2.70	.05	31	16.6	2.85[c]	.06
50 ppm	49	14.6	2.74	.05	37	16.2	2.84[c]	.06
500 ppm	48	14.4	2.72[a]	.05	39	17.5	2.91[bc]	.06
5000 ppm	26	17.7	2.85[a]	.07	21	26.4	3.29[bd]	.08
Aorta								
0 ppm					28	13.8	2.67	.07
50 ppm	50	10.9	2.44[ac]	.05	39	13.6	2.66[b]	.06
500 ppm					39	12.2	2.56	.06
5000 ppm	25	13.6	2.64[d]	.07	21	12.2	2.56	.08
Testes								
0 ppm					12	9.8	2.36	.11
50 ppm					19	10.8	2.46	.08
500 ppm					19	10.8	2.46	.08
5000 ppm					10	11.4	2.51	.11

[ab]Values for parities within treatment and tissue (same line) which have different superscripts differ significantly ($P<.05$).
[cd]Values for treatments which have different superscripts in the columns within tissues differ significantly ($P<.05$).

diet had lower hepatic zinc stores at 21 days of age compared to the neonate's stores (table 22). These data would indicate that sows on this excessive level of zinc do not have a placental mechanism to prevent high levels of zinc from crossing to the fetuses, but by 21 days of age a homeostatic mechanism has been able to reduce the high hepatic zinc stores found in the neonate. Only second parity pigs from sows receiving no supplemental zinc in their diet had higher zinc stores than first parity pigs (table 23). This is not what might be expected since these sows had been fed an unsupplemented diet since weighing approximately 30 kg. However, pigs from sows in this unsupplemented group still had lower zinc stores than pigs from sows on the other treatments, and pigs from sows fed 5000 ppm added zinc had higher stores than pigs whose dams were supplemented with 50 or 500 ppm zinc. Schlicker and Cox (1968) reported that hepatic zinc stores were not significantly higher for rats whose dams were fed 4000 ppm zinc until the fetus was 20 days of age although the total fetal zinc concentration was higher in an 18 day old fetus than in the fetus from a dam receiving the basal diet. Numerous researchers have reported that feeding additional zinc to animals increases hepatic zinc stores.

In this study serum zinc was reduced in pigs that were 21 days of age compared to the newborn pig in all teatments (.89 µg/ml vs .61 µg/ml). Pigs whose dam's received 5000 ppm zinc had significantly higher circulating zinc levels than those whose dams were fed the other dietary treatments regardless of age (.69, .56, .60 µg/ml vs 1.5 µg/ml). The concentration of zinc in the renal tissue was increased in pigs whose dams were fed the highest level of zinc compared to pigs from dams on the other treatments. Parity did not affect the zinc

stores in the kidneys. The pancreas which utilizes zinc in insulin production was found to contain the lowest level of zinc in first parity pigs when the dam had received 0 ppm supplementation and in second parity pigs from dams on the 0 and 50 ppm zinc treatments. Zinc in the pancreas was intermediate in concentration of first and second parity pigs from sows fed 500 ppm zinc and was highest in both parities in pigs from sows fed 5000 ppm. The sows fed 5000 ppm zinc had higher zinc concentrations in the pancreas than did sows fed the other dietary treatments (Hill et al. 1981b). Only pigs whose dams were fed 50 ppm showed a parity effect for this parameter, and the concentration was lower in pigs from the second parity. Eltohamy et al. (1980) observed the pancreas from Leghorn cocks fed 3000 or 4000 ppm zinc to have marked changes in the acini and islets. The acinar cells had fewer zymogen and cytoplasmic granules, and the connective tissue between acini was increased. In this study, the zinc contained in the esophagus was not affected by treatment in the first parity but was significantly higher in pigs from dams on the highest supplementation level in the second parity. Also, the zinc concentration was increased in pigs from sows fed either 500 or 5000 ppm in the second parity group compared to the first parity. Aortas were removed from all pigs in the second parity but only those whose dams received 50 or 5000 ppm zinc in the first parity. Zinc was higher in the aorta of the first parity pigs whose dams received the highest supplementation, but there was no treatment effect on the aortas from second parity pigs. However, zinc was higher in the aortas of second parity pigs whose dams were fed 50 ppm supplemental zinc compared to first parity pigs.

Because of the need for zinc for the male in reproduction, testes from second parity males were analyzed for zinc but no treatment differences were found. Eltohamy et al. (1980) reported that Leghorn cocks fed 8000 ppm zinc had decreased testes weights, reduced seminiferous tubule size and hyperplasia in the interstitial cells. Spermatogenesis was delayed and only the Sertoli cells and spermatogonia appeared to be in good condition.

Second parity pigs whose dams received 5000 ppm supplemental zinc had lower hepatic copper stores than first parity pigs, but the reverse was true for pigs whose dams received no additional zinc (table 24). As expected because of the inverse relationship between zinc and copper, pigs whose dams were on the highest zinc treatment had the lowest hepatic copper stores. Among the second parity pigs, those from dams on the unsupplemented diet had higher liver copper stores than pigs whose dams received 50 or 500 ppm supplementation of zinc. This follows the same pattern as observed in their dams (Hill et al., 1981b). Ritchie et al. (1963) reported that after 16 weeks on their respective dietary treatments, pigs feed 100 ppm zinc had hepatic copper stores similar to pigs fed either 100 ppm zinc and 125 ppm copper or 1.3% calcium. These three treatment's hepatic copper stores were significantly lower than those found in pigs fed 125 ppm copper, 250 ppm copper or 100 ppm zinc and 250 ppm copper. Cox and Hale (1962) did not observe reduced hepatic copper stores when 2000 or 4000 ppm zinc were fed to pigs. When 4000 ppm were fed to pregnant rats, 15 and 16 day old fetuses did not have reduced copper but the concentration of copper was reduced in the total fetus and fetal liver of 18 day post-conception offspring.

Table 24. Effect of treatment and parity on the concentration of copper in tissues of offspring (ppm, wet basis)

Dam's treatment and tissue	Parity 1 N	Mean	Mean ln	SE	Parity 2 N	Mean	Mean ln	SE
Liver								
0 ppm	43	55.6	3.95^{ac}	.04	31	64.6	4.13^{bc}	.05
50 ppm	50	47.5	3.81^{c}	.04	39	55.5	3.96^{d}	.05
500 ppm	50	52.1	3.89^{c}	.04	39	52.5	3.89^{d}	.05
5000 ppm	26	5.6	1.13^{ad}	.06	21	1.3	.79^{be}	.06
Heart								
0 ppm	43	3.0	1.38^{c}	.04	31	2.8	1.33^{c}	.05
50 ppm	49	3.0	1.38^{c}	.04	39	2.8	1.33^{c}	.05
500 ppm	50	3.1	1.41^{c}	.04	39	2.8	1.32^{c}	.05
5000 ppm	26	1.9	1.04^{d}	.06	21	1.5	.89^{d}	.06
Kidney								
0 ppm	43	6.0	1.83^{c}	.04	31	5.3	1.74^{cd}	.05
50 ppm	50	5.1	1.75^{c}	.04	38	4.2	1.60^{c}	.05
500 ppm	50	5.3	1.75^{c}	.04	39	5.4	1.77^{cd}	.05
5000 ppm	26	11.9	2.24^{ad}	.06	21	6.6	1.91^{bd}	.06
Pancreas								
0 ppm	43	1.2	.78^{c}	.04	29	1.1	.75^{c}	.05
50 ppm	49	1.1	.75^{c}	.04	39	1.1	.72^{c}	.05
500 ppm	47	1.2	.77^{c}	.04	39	1.0	.69^{cd}	.05
5000 ppm	26	.8	.58^{d}	.06	21	.7	.50^{d}	.06
Esophagus								
0 ppm	42	1.4	.86^{c}	.04	31	1.2	.77^{c}	.05
50 ppm	48	1.1	.70^{d}	.04	37	1.1	.76^{c}	.05
500 ppm	48	.9	.63^{ade}	.04	39	1.2	.78^{bc}	.05
5000 ppm	26	.9	.50^{e}	.06	21	1.0	.57^{d}	.06
Aorta								
0 ppm					28	.80	.58^{c}	.06
50 ppm	50	.81	.59	.04	39	.82	.59^{c}	.05
500 ppm					39	.82	.59^{c}	.05
5000 ppm	25	.66	.49	.06	21	.44	.36^{d}	.06
Testes								
0 ppm					12	1.7	.98^{cd}	.08
50 ppm					19	1.8	1.02^{c}	.07
500 ppm					19	1.8	1.00^{c}	.07
5000 ppm					10	1.1	.71^{d}	.09

^{ab}Values for parities within treatment and tissue (same line) which have different superscripts differ significantly ($P<.05$).

^{cde}Values for treatments which have different superscripts in columns within tissues differ significantly ($P<.05$).

Because pigs whose dams received 5000 ppm supplemental zinc had very low hepatic copper concentrations, it was theorized that they would become copper-deficient if placed on a copper-deficient diet. A dried skim milk basal diet was supplemented with 0, 5 or 10 ppm copper and fed to neonatal pigs from sows fed 5000 ppm zinc. The results of this experiment are provided in Appendix Tables 39, 40 and 41.

Because copper is required by some of the enzymes of the electron-transport chain, there is a minimum concentration necessary for sufficient cardiac function. In both parities, pigs whose dams were fed 5000 ppm supplemental zinc had lower copper concentrations in the heart than did pigs whose dams were on the other treatments. As reported for the dams (Hill et al., 1981b), first parity pigs from sows fed 5000 ppm zinc had a higher concentration of copper in the kidneys than did pigs from the other treatments. However, the second parity pigs from these sows had significantly lower copper in the kidneys than did the first parity pigs. As a result, the copper concentration in this tissue from pigs born to sows on the highest zinc treatment was higher than that of pigs whose dams received 50 ppm additional zinc but did not differ from pigs whose dams were fed the other dietary treatments. When Magee and Matrone (1960) fed 7500 ppm supplemental zinc to rats, they noted that a higher percent of the absorbed ^{64}Cu was present in the urine than with rats fed the control diet. The reverse was true for the absorbed ^{64}Cu found in kidney tissue. Copper concentrations in the pancreas were higher in pigs from sows fed 0, 50 or 500 ppm supplemented zinc in the first parity and from sows fed 0 or 50 ppm supplemental zinc in the second parity. The concentration of copper in the pancreas within treatment was not affected by parity (table

24). Copper in the esophagus of first parity pigs was lowest in pigs whose dams had received 5000 ppm supplemental zinc, intermediate in pigs from dams fed 50 or 500 ppm zinc and highest in pigs whose dams were unsupplemented. Pigs from sows fed 500 ppm had increased copper concentrations in the second parity compared to the first. Therefore, pigs from sows fed 0, 50 or 500 ppm zinc had higher copper in this tissue than pigs from sows on the highest supplemented group. Copper tended to be lower in the aortas of first parity pigs from sows fed 5000 ppm than from pigs whose dams received 50 ppm zinc, but by second parity, copper was significantly lower for pigs from sows from this highest supplemented group compared to all others. Lysyl oxidase, an enzyme essential for collagen cross-linking in the aorta, requires copper to function. Its reduced activity is associated with ruptured aortas in copper-deficient animals. The reduced copper concentration in the aorta of pigs from sows fed 5000 ppm zinc could reflect reduced activity of this enzyme. The copper concentration in the testes was higher from pigs whose dams received 50 or 500 than from pigs whose dams received 5000 ppm supplemental zinc. Serum copper was significantly higher in pigs at 21 days of age than at 1 day of age (1.69 µg/ml vs .55 µg/ml) and in pigs from dams fed 0 ppm zinc compared to 5000 ppm zinc (2.04 µ g/ml vs .09 µg/ml). Hemoglobin concentration was also reduced in pigs whose dam received the highest supplementation of zinc (9.6, 10.5, 10.0 vs 8.4 g/dl).

Only tissues from the first parity pigs were analyzed for calcium, phosphorus and manganese. Calcium was higher in the liver of pigs whose dams received 5000 ppm zinc but was not affected by dam's treatment in the heart, kidney or pancreas (table 25). Stewart and Magee

Table 25. Effect of dam's treatment on concentration of calcium, manganese and phosphorus in tissue of offspring (ppm, wet basis)

Dam's	Liver			Heart			Kidney			Pancreas		
treatment	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
Calcium												
0 ppm	43	34.7[a]	1.9	43	46.7	1.9	43	64.1	1.9	43	114.6	1.9
50 ppm	50	34.5[a]	1.8	49	40.8	1.8	50	68.1	1.8	49	114.1	1.8
500 ppm	50	35.2[a]	1.8	50	40.8	1.8	50	65.7	1.8	48	119.3	1.8
5000 ppm	26	43.0[b]	2.5	26	43.1	2.5	26	66.6	2.5	26	116.8	2.5
Manganese												
0 ppm	43	2.93[ab]	.17	43	.40	.17	43	1.37	.17	43	2.83	.17
50 ppm	50	2.38[a]	.16	49	.49	.16	50	1.43	.16	48	2.93	.16
500 ppm	50	2.45[a]	.16	50	.35	.16	50	1.38	.16	47	2.68	.16
5000 ppm	26	3.46[b]	.22	26	.34	.22	26	1.33	.22	24	3.05	.22
Phosphorus												
0 ppm	43	3591	143	43	2081	143	43	3096	143	43	3289	143
50 ppm	50	3179	133	49	2468	135	50	3361	133	49	2986	135
500 ppm	50	3150	133	50	2240	133	50	2848	133	49	3075	135
5000 ppm	26	2971	185	26	1865	185	26	2578	185	26	3423	185

[ab]Means with different superscripts in the same column within elements differ significantly ($P<.05$).

(1964) reported that increased levels of dietary zinc resulted in significant decreases in bone calcium and phosphorus deposition. When calcium was also supplemented in the diet (.4, .8 or 1.2%), an improvement in bone calcium resulted, and the decreased bone phosphorus was partially alleviated. Phosphorus supplementation (.4, .8 or 1.2%) of the high zinc diets (7500 ppm) had no beneficial effect on the bone phosphorus levels. Calcium retention was reduced in rats fed 7500 ppm, and the amount retained decreased as the balance trial progressed (Stewart and Magee, 1964). Hsu et al. (1975) did not observe an effect on the calcium and phosphorus contents of the humerus and femur from pigs fed 57 ppm zinc, .7% calcium, .6% phosphorus; 4000 ppm zinc, .7% calcium, .6% phosphorus or 57 ppm zinc, 1.1% calcium, 1.0% phosphorus. The known interaction between calcium and zinc has demonstrated that high dietary calcium can decrease levels of zinc in the body (Hsu et al., 1975) but the reverse does not appear to occur.

Manganese was higher in the liver of pigs whose dams received 5000 ppm supplemental zinc than in the livers of pigs from dams fed 50 or 500 ppm zinc. The dam's dietary treatment did not influence manganese concentration in the other organs which were studied. Like calcium, the increased deposition of manganese in the liver of the offspring of sows fed high levels of zinc is contradictory to the expected trend.

Age of the pig and dietary treatment of the dam significantly affected the phosphorus content of the liver. Pigs from sows fed 0, 50 or 500 ppm zinc had higher phosphorus levels in the liver at 21 days than 1 day of age, but pigs from sows fed 5000 ppm dietary zinc had significantly lower levels of this element in the liver (table 26). At one day of age, phosphorus was highest in the liver of pigs from dams

Table 26. Effect of dam's treatment and age on concentration of phosphorus in the liver, 1st parity (ppm wet basis)

Dam's	Age					
	1 day			21 days		
treatment	N	Mean	SE	N	Mean	SE
0 ppm	30	3320^{ac}	35.7	21	3880^{bc}	42.7
50 ppm	30	2920^{ad}	35.7	26	3410^{bd}	38.4
500 ppm	26	2990^{ad}	38.4	24	3350^{bd}	39.3
5000 ppm	14	3060^{ad}	52.3	11	2810^{be}	59.0

^{ab}Values on the same line with different superscripts are significantly different ($P<.05$).

^{cde}Values in the same column with different superscripts are significantly different ($P<.05$).

unsupplemented with zinc. At 21 days of age, this group still had the highest phosphorus level while livers from pigs whose dams were fed 50 or 500 ppm were intermediate and those from sows fed 5000 ppm were lowest. Age or treatment did not affect the phosphorus levels in the other organs (table 25). Stewart and Magee (1964) reported that phosphorus retention was reduced when high levels of zinc were fed to rats, but bone phosphorus levels were not altered in the pig when high zinc was fed (Hsu et al., 1975). Unlike calcium and manganese, phosphorus concentration in the liver of 21 day old pigs appears to be depressed by high dietary zinc.

The mineral concentrations in organs of offspring from sows fed varying levels of zinc do not appear to always be the same as when animals have been fed the zinc directly. The ability of the placenta to selectively allow reduced levels of zinc to cross unless affected by extremely high circulating levels of this element may account for the similarity of zinc depositions in organs from pigs whose dams were fed 0, 50 or 500 ppm zinc. The reduced copper found in pigs from sows fed the high zinc was expected but the interactions which occured with the other elements need further elucidation.

SECTION IV

Effect of Dietary Zinc Levels on Mineral Concentration in Milk

Introduction

The mammalian neonate's requirement for nutrients must be met by dietary sources or body stores, and usually the dietary source is supplied by milk from the dam. It is well documented that the mineral concentration in colostrum differs from that found in milk produced at later stages of lactation (Perrin, 1955; Ullrey et al., 1974; Earle and Stevenson, 1965; Underwood, E.J., 1977; Johnson and Evans, 1978; Pond and Houpt, 1978). Some mineral elements in the milk are influenced more by diet composition than are others as a result of mammary transfer from the plasma (Linzell, 1968). For example, calcium, phosphorus, iron and copper are generally thought to be resistant to the influence of dietary levels (Pond, et al., 1965; Underwood, E.J., 1977; Pond and Houpt, 1978) while zinc (Miller, et al., 1965; Earle and Stevenson, 1965) and manganese (Plumlee, et al., 1956) can be increased in milk by increasing the dietary levels of the dam. Mutch and Hurley (1974) have shown that low levels of dietary zinc influence the levels of zinc found in the milk. Utilizing radioactive labeling studies, Johnson and Evans (1980) noted that dietary zinc is utilized in milk secretion more readily than zinc found in body stores. In addition to the amount of an element present, the bioavailability of each element may be influenced by other components of the milk (Johnson and Evans, 1978; Ainscough et al. 1980; Cousins and Smith, 1980; Evans and Johnson, 1980; Lönnerdal et al., 1980). Percent ash in milk is increased and milk

yield is decreased by energy undernutrition during lactation (Rook and Witter, 1968).

The objective of this study was to evaluate the influence of a range of zinc concentrations in the diet on the concentration of zinc, copper, iron, calcium, phosphorus and magnesium in the milk of first and second parity sows for the first three weeks of lactation.

Experimental Procedure

Experimental animals. Sixty crossbred and purebred Yorkshire gilts averaging 30 kg body weight were blocked by the date they were farrowed into four treatment groups. Thus, each treatment group of five animals per pen was represented in each of the three blocks. The gilts were housed in a total confinement facility with slotted floors, cast-iron automatic waterers and wood/non-galvanized metal self-feeders. The pens were 4.27 meters by 1.21 meters from 30 kg until approximately 60 kg body weight and 4.87 meters by 1.21 meters until approximately 100 kg of body weight.

After reaching approximately 100 kg body weight, the gilts were moved from the total confinement facility to dirt or concrete lots. Gilts were moved by group to individual crates in the farrowing facility when gestational length was approximately 110 days for at least one of the gilts. After farrowing and weaning, sows were housed individually or by treatment group in a confinement facility with partial or total slats. A basal corn-soybean meal diet (grower) which met NRC requirements (1979) was fed ad libitum until the lightest animals reached approximately 60 kg (table 1). The same diet was also fed ad libitum during lactation. From 60 kg until farrowing, a developer diet was fed (table 1). After reaching approximately 100 kg body weight,

the gilts were limit fed 1.75 kg to 2.75 kg of feed per day depending on climatic conditions. Water was available ad libitum throughout the study. The basal diet was supplemented with zinc from feed grade zinc oxide at the following levels: 0, 50, 500 and 5000 ppm added zinc (table 2). Laboratory analyses of the diets revealed that NRC mineral requirements were met (table 3).

Gilts were field-mated between seven and eight months of age for the first parity. Sows were hand-mated for their second parity at the first estrus following weaning.

Milk sampling and analyses. During parturition or within 24 hours after the onset of parturition, colostrum samples (0 week) were obtained. Milk samples were collected at 7 days (1 week), 14 days (2 weeks) and 21 days (3 weeks) after parturition. Each sample consisted of milk from one or more mammary sections and was collected after the mammary glands were washed with 70% ethanol and an intramuscular injection of oxytocin was given. Milk was collected and stored in acid-washed vials at -20 C until analyzed.

Duplicate samples were wet digested in a mixture of nitric and perchloric acids and diluted with deionized-distilled water as necessary for analyses. For calcium and magnesium determinations, strontium chloride was used to reduce matrix interference. Copper, iron, zinc, calcium and magnesium determinations were made by atomic absorption spectrophotometry (IL-453, Instrumentation Laboratory, Lexington, MA). Phosphorus determinations were made by use of the Gomori modification of the Fiske and Subbarow procedure (Gomori, 1942).

Statistical analyses. A modified version of Kolmogorov-Smirnov D-statistic was utilized to test for the probability of nonnormality. Because the data was distinctly nonnormal, a natural logarithm transformation was utilized to ensure near normality of distribution for the transformed variables (Gill, 1978). Gill (1978) points out that the logarithm of the measurement may be more normally distributed than the measurement itself. Because the natural log of zero is indeterminant and the natural log of one is zero, one was added to each observation before the observation was converted to a natural log value. Analysis of variance was performed using the General Linear Models procedure of the Statistical Analysis System maintained at Wayne State University. A procedure involving Bonferroni t statistics was utilized for comparisons among means (Gill, 1978).

Because the animals were assigned randomly to the dietary treatments, grouped by date farrowed into blocks and measured for trend at four sampling times within two parities, a split-plot design was utilized. This design allows for the separation of random error into variation among and variation within subjects. Analysis of variance revealed a significant interaction between treatment x time, thus indicating nonparallel trends in response over time to the dietary treatments of added levels of zinc. Therefore, comparisons of treatments within sampling periods, of sampling periods within the treatment and of parities within treatment and sampling period were made. After decimal points were removed by multiplication and a natural logarithm transformation of the data was performed, a modified version of the Kolmogorov-Smirnov D-statistic was utilized to test for the probability of nonnormality. The residuals of the natural log transformed iron

data were considered to be normally distributed. The residuals of the other natural log transformed variables were still considered nonnormal but at a reduced probability. Numerous efforts did not reveal a more desirable transformation. Although the accuracy of the probability statements was improved, natural log transformed data were difficult to interpret. For this reason, the mean of the original data (not transformed) are also given in the tables.

Results and Discussion

Comparison of dietary treatment effects within stage of lactation. The concentration of iron, zinc and magnesium in colostrum was not affected by dietary treatment (table 27). The colostrum collected from sows receiving 0 or 5000 ppm added zinc contained less calcium ($P<.01$), than colostrum from sows on the other treatments, but phosphorus was only depressed by the 5000 ppm added zinc treatment. Copper was highest in the colostrum of sows and gilts receiving 0 and 500 ppm added dietary zinc and was depressed ($P<.01$) when 5000 ppm of zinc was fed compared to other treatments. The effect of excessive dietary zinc on the level of copper in tissues and enzymes requiring copper has been reported (VanReen, 1953; Magee and Matrone, 1960; Cox and Harris, 1960; Ott et al., 1966a,b; Chavapil and Misiorowski, 1980). However, this depressing effect on the copper concentration in milk has not been reported.

When milk collected at 7 and 14 days post-partum was compared, copper was lower and zinc was higher in milk from sows fed the highest dietary zinc treatment (tables 28 and 29). Calcium, magnesium and phosphorus concentrations were not affected by treatment in the 1 or 2 week milk samples. Although the iron concentration in the milk was not

Table 27. The concentration of minerals in colostrum (0 week) from sows fed 0, 50, 500, 5000 ppm Zn

		Cu			Fe		
Treatment	N	Mean μg/ml	Transformed mean[a]	SE	Mean μg/ml	Transformed mean[a]	SE
0 ppm	20	3.830	3.55[d]	.08	2.165	3.09	.07
50 ppm	23	2.731	3.22[e]	.08	2.074	3.04	.07
500 ppm	22	3.036	3.29[de]	.08	2.005	3.00	.07
5000 ppm	15	.400	1.52[f]	.10	2.280	3.14	.09
		Zn			Ca		
	N	Mean μg/ml	Transformed mean[a]	SE	Mean %	Transformed mean[b]	SE
0 ppm	20	14.77	4.96	.08	.070	2.03[d]	.04
50 ppm	23	14.60	4.88	.07	.088	2.26[e]	.04
500 ppm	22	13.91	4.85	.07	.084	2.22[e]	.04
5000 ppm	15	19.35	5.13	.09	.067	2.01[d]	.05
		Mg			P		
	N	Mean μg/ml	Transformed mean[c]	SE	Mean %	Transformed mean[b]	SE
0 ppm	20	98.25	4.58	.05	.12	2.58[d]	.05
50 ppm	23	104.90	4.62	.05	.13	2.65[d]	.05
500 ppm	22	102.57	4.62	.05	.13	2.60[d]	.05
5000 ppm	15	101.80	4.65	.06	.12	2.39[e]	.06

[a]Individual values were multiplied by 10, had 1 added and were then transformed to their natural logarithm for data analysis.

[b]Individual values were multiplied by 100, had 1 added and were then transformed to their natural logarithm for data analysis.

[c]Individual values had 1 added and were transformed to their natural logarithm for data analysis.

[def]Means in the same column for the same element with different superscripts differ ($P<.01$).

Table 28. Concentration of minerals in first week milk from sows fed 0, 50, 500, 5000 ppm Zn

	Cu				Fe		
Treatment	N	Mean µg/mg	Transformed mean[a]	SE	Mean µg/ml	Transformed mean[a]	SE
0 ppm	20	1.52	2.75[a]	.08	2.16	3.01	.07
50 ppm	23	1.34	2.63[a]	.08	1.87	2.93	.07
500 ppm	22	1.45	2.72[a]	.08	1.87	2.93	.07
5000 ppm	11	.24	1.02[b]	.11	2.29	3.12	.10

	Zn				Ca		
	N	Mean µg/ml	Transformed mean[a]	SE	Mean %	Transformed mean[b]	SE
0 ppm	20	6.52	4.17[a]	.08	.19	2.95	.04
50 ppm	23	5.98	4.03[a]	.07	.19	3.01	.04
500 ppm	22	6.78	4.20[a]	.07	.19	2.95	.04
5000 ppm	11	10.27	4.62[b]	.10	.20	3.01	.06

	Mg				P		
	N	Mean µg/ml	Transformed mean[c]	SE	Mean %	Transformed mean[b]	SE
0 ppm	20	123.43	4.81	.05	.14	2.72	.05
50 ppm	23	119.10	4.77	.05	.14	2.69	.05
500 ppm	22	115.10	4.74	.05	.14	2.70	.05
5000 ppm	11	128.08	4.84	.07	.15	2.74	.07

[a]Individual values were multiplied by 10, had 1 added and were then transformed to their natural logarithm for data analysis.

[b]Individual values were multiplied by 100, had 1 added and were then transformed to their natural logarithm for data analysis.

[c]Individual values had 1 added and were transformed to their natural logarithm for data analysis.

[de]Means in the same column for the same element with different superscripts differ ($P<.01$).

Table 29. The concentration of minerals in second week milk from sows fed 0, 50, 500, 5000 ppm Zn

	Cu				Fe		
Treatment	N	Mean µg/ml	Transformed mean[a]	Se	Mean µg/ml	Transformed mean[a]	SE
0 ppm	20	1.29	2.62[d]	.08	2.07	3.05[d]	.07
50 ppm	22	1.20	2.54[d]	.08	1.36	2.67[d]	.07
500 ppm	21	1.14	2.51[d]	.08	1.83	2.92[d]	.07
5000 ppm	9	.28	1.27[e]	.13	2.12	3.07[e]	.11

	Zn				Ca		
	N	Mean µg/ml	Transformed mean[a]	SE	Mean %	Transformed mean[b]	SE
0 ppm	20	6.81	4.22[d]	.08	.20	3.05	.04
50 ppm	22	6.43	4.16[d]	.07	.20	3.02	.04
500 ppm	21	6.61	4.18[d]	.07	.20	3.02	.04
5000 ppm	9	10.66	4.65[e]	.11	.24	3.18	.07

	Mg				P		
	N	Mean µg/ml	Transformed mean[c]	SE	Mean %	Transformed mean[b]	SE
0 ppm	20	128.45	4.85	.05	.15	2.79	.05
50 ppm	22	121.20	4.80	.05	.14	2.68	.05
500 ppm	21	122.03	4.78	.05	.15	2.75	.05
5000 ppm	9	134.8	4.89	.08	.15	2.78	.08

[a]Individual values were multiplied by 10, had 1 added and were then transformed to their natural logarithm for data analysis.

[b]Individual values were multiplied by 100, had 1 added and were then transformed to their natural logarithm for data analysis.

[c]Individual values had 1 added and were transformed to their natural logarithm for data analysis.

[de]Means in the same column for the same element with different superscripts differ ($P<.01$).

affected by 7 days of lactation, it was significantly lower at 14 days in milk from sows fed 50 ppm added zinc. Pond et al. (1965) observed a similar depression at 14 days post-partum.

Calcium, magnesium and phosphorus concentrations in milk samples collected at 21 days were not affected by dietary treatment (table 30). Zinc was lower in milk from sows fed no added zinc when compared to sows fed 5000 ppm. Copper was depressed at this stage of lactation in the treatment receiving the highest zinc compared to other treatments. Only iron in milk from sows and gilts receiving 500 and 5000 ppm zinc differed at 3 weeks post partum.

Comparison of effect of stage of lactation within dietary treatment. When no additional zinc was added to the diet (0 ppm), the concentration of copper and zinc was higher in the colostrum than in milk obtained later in lactation (table 31). The reverse was observed with calcium and magnesium. The level of iron in the milk from sows supplemented with 0 ppm zinc at all stages of lactation was not different. Only at 14 days of lactation was the mean concentration of phosphorus in the milk significantly greater ($P<.01$) than the colostrum level. Pond et al (1965) reported that sows receiving 150 ppm of iron and 60 ppm of zinc in their diets had 1.5, 1.34 and 1.47 mg/liter and 4.93, 4.53, 5.09 mg/kg on a fresh basis of iron and zinc, respectively, in milk samples at 1, 2 and 3 weeks post-farrowing. Earle and Stevenson (1965) reported that zinc in colostrum ranged from 9 to 24 mg/kg of whole milk for sows fed on diets unsupplemented with zinc (47 ppm gestation; 73 ppm lactation) and 8 to 28 mg/kg for sows whose diets had been supplemented with 100 ppm zinc. Our values (table 31) are slightly higher for zinc than those of Pond et al. (1965). These differences

Table 30. The concentration of minerals in third week milk from sows fed 0, 50, 500, 5000 ppm Zn

	Cu				Fe		
Treatment	N	Mean µg/ml	Transformed mean[a]	SE	Mean µg/ml	Transformed mean[a]	SE
0 ppm	20	1.27	2.59[d]	.08	1.81	2.90[de]	.07
50 ppm	22	1.17	2.52[d]	.08	1.75	2.90[de]	.07
500 ppm	20	1.09	2.47[d]	.08	1.63	2.77[d]	.07
5000 ppm	9	.21	1.03[e]	.13	2.67	3.22[e]	.11

	Zn				Ca		
	N	Mean µg/ml	Transformed mean[a]	SE	Mean %	Transformed mean[b]	SE
0 ppm	20	6.56	4.19[a]	.08	.21	3.09	.04
50 ppm	22	7.43	4.31[de]	.07	.22	3.14	.04
500 ppm	20	7.81	4.35[de]	.08	.22	3.13	.04
5000 ppm	9	10.81	4.65[e]	.11	.24	3.21	.07

	Mg				P		
	N	Mean µg/ml	Transformed mean[c]	SE	Mean %	Transformed mean[b]	SE
0 ppm	20	130.5	4.87	.05	.15	2.71	.05
50 ppm	22	127.3	4.84	.05	.15	2.75	.05
500 ppm	20	134.98	4.90	.05	.16	2.80	.05
5000 ppm	9	142.36	4.96	.09	.16	2.84	.08

[a]Individual values were multiplied by 10, had 1 added and were then transformed to their natural logarithm for data analysis.

[b]Individual values were multiplied by 100, had 1 added and were then transformed to their natural logarithm for data analysis.

[c]Individual values had 1 added and were transformed to their natural logarithm for data anlaysis.

[de]Means in the same column for the same element with different superscripts differ ($P<.01$).

Table 31. The concentration of minerals in 0, 1, 2, 3 week milk of sows fed 0 ppm added Zn

		Cu			Fe		
Time(wk)	N	Mean µg/ml	Transformed mean[a]	SE	Mean µg/ml	Transformed mean[a]	SE
0	20	3.83	3.55[d]	.08	2.17	2.09	.07
1	20	1.52	2.75[e]	.08	2.16	3.07	.07
2	20	1.29	2.62[e]	.08	2.07	3.05	.07
3	20	1.27	2.59[e]	.08	1.81	2.90	.07
		Zn			Ca		
	N	Mean µg/ml	Transformed mean[c]	SE	Mean %	Transformed mean[b]	SE
0	20	14.77	4.96[d]	.08	.07	2.03[d]	.04
1	20	6.52	4.17[e]	.08	.19	2.95[e]	.04
2	20	6.81	4.22[e]	.08	.20	3.05[e]	.04
3	20	6.56	4.19[e]	.08	.21	3.09[e]	.04
		Mg			P		
	N	Mean µg/ml	Transformed mean[c]	SE	Mean %	Transformed mean[b]	SE
0	20	98.25	4.58[d]	.05	.12	2.58[d]	.05
1	20	123.43	4.81[e]	.05	.14	2.72[de]	.05
2	20	128.45	4.85[e]	.05	.15	2.79[e]	.05
3	20	130.59	4.87[e]	.05	.15	2.71[de]	.05

[a]Individual values were multiplied by 10, had 1 added and were then transformed to their natural logarithm for data analysis.

[b]Individual values were multiplied by 100, had 1 added and were then transformed to their natural logarithm for data analysis.

[c]Individual values had 1 added and were transformed to their natural logarithm for data anlaysis.

[de]Means in the same column for the same element with different superscripts differ ($P<.01$).

could be due to differing zinc stores of the dam, contamination of the samples or differing sensitivity of the analytical techniques. In our laboratory, zinc recoveries are 85% or higher.

When 50 ppm zinc was added to the diet of sows and gilts, copper levels in colostrum were higher ($P<.05$) than at any other measured stage of lactation (table 32). Iron was lowest in the milk at 14 days post-farrowing. Zinc concentrations in colostrum were significantly higher than at other times; second and third week milk zinc levels were lower ($P<.05$) than first week levels. Phosphorus level in milk was not affected by stage of lactation in this treatment group.

As with the dietary treatment of 0 ppm added zinc, copper and zinc were increased ($P<.05$) in colostrum and iron was not affected when 500 ppm zinc was added to the diet (table 33). However, calcium and magnesium were lower in colostrum than at other times and higher in milk collected at 14 and 21 days than at 7 days of lactation. Phosphorus was higher in 14 and 21 day milk than in colostrum. Earle and Stevenson (1965) found that adding 100 ppm zinc to the diet of lactating sows (145 ppm zinc total in diet) resulted in 49% increase in zinc in whole milk at 35 days of lactation compared with milk from unsupplemented sows (10.3 vs 6.9 mg/kg whole milk). Pond *et al.* (1965) reported that when sows were fed approximately 60 ppm of zinc during a 3 week lactation, the mean value for the concentration of zinc in the milk was 4.94 mg/kg on a fresh basis.

Copper was higher in colostrum of sows fed 5000 ppm added zinc than in milk at 7 and 21 days post-farrowing (table 34). Colostrum zinc was higher and colostrum calcium and phosphorus lower ($P<.01$) than at other stages of lactation. Iron in the milk was not affected by the

Table 32. The concentration of minerals in 0, 1, 2, 3 week milk of sows fed 50 ppm added Zn

		Cu			Fe		
Time(wk)	N	Mean μg/ml	Transformed mean[a]	SE	Mean μg/ml	Transformed mean[a]	SE
0	23	2.73	3.22[d]	.08	2.07	3.04[d]	.07
1	23	1.34	2.63[e]	.08	1.87	2.93[d]	.07
2	22	1.20	2.54[e]	.08	1.36	2.67[e]	.07
3	22	1.17	2.52[e]	.08	1.75	2.90[d]	.07
		Zn			Ca		
	N	Mean μg/ml	Transformed mean[a]	SE	Mean %	Transformed mean[b]	SE
0	23	14.60	4.88[d]	.07	.09	2.26[d]	.04
1	23	5.98	4.03[e]	.07	.19	3.01[e]	.04
2	22	6.43	4.16[ef]	.07	.20	3.02[e]	.04
3	22	7.43	4.31[f]	.07	.22	3.14[e]	.04
		Mg			P		
	N	Mean μg/ml	Transformed mean[c]	SE	Mean %	Transformed mean[b]	SE
0	23	104.9	4.62[d]	.05	.13	2.65	.05
1	23	119.1	4.77[e]	.05	.14	2.69	.05
2	23	121.2	4.80[e]	.05	.14	2.68	.05
3	23	127.3	4.84[e]	.05	.15	2.75	.05

[a]Individual values were multiplied by 10, had 1 added and were then transformed to their natural logarithm for data analysis.

[b]Individual values were multiplied by 100, had 1 added and were then transformed to their natural logarithm for data analysis.

[c]Individual values had 1 added and were transformed to their natural logarithm for data anlaysis.

[def]Means in the same column for the same element with different superscripts differ (P<.05).

Table 33. The concentration of minerals in 0, 1, 2, 3 week milk of sows fed 500 ppm added Zn

		Cu			Fe		
Time(wk)	N	Mean µg/ml	Transformed mean[a]	SE	Mean µg/ml	Transformed mean[a]	SE
0	22	3.04	3.29[d]	.08	2.01	3.00	.07
1	22	1.45	2.72[e]	.08	1.87	2.93	.07
2	21	1.14	2.51[e]	.08	1.83	2.92	.07
3	20	1.09	2.47[e]	.08	1.63	2.77	.07
		Zn			Ca		
	N	Mean µg/ml	Transformed mean[a]	SE	Mean %	Transformed mean[b]	SE
0	22	13.91	4.85[d]	.07	.08	2.22[d]	.04
1	22	6.78	4.20[e]	.07	.19	2.95[e]	.04
2	21	6.61	4.18[e]	.07	.20	3.02[ef]	.04
3	20	7.81	4.35[e]	.08	.22	3.13[f]	.04
		Mg			P		
	N	Mean µg/ml	Transformed mean[c]	SE	Mean %	Transformed mean[b]	SE
0	22	102.57	4.62[d]	.05	.13	2.60[d]	.05
1	22	115.10	4.74[e]	.05	.14	2.70[de]	.05
2	21	122.03	4.78[e]	.05	.15	2.75[e]	.05
3	20	134.98	4.90[f]	.05	.16	2.80[e]	.05

[a]Individual values were multiplied by 10, had 1 added and were then transformed to their natural logarithm for data analysis.

[b]Individual values were multiplied by 100, had 1 added and were then transformed to their natural logarithm for data analysis.

[c]Individual values had 1 added and were transformed to their natural logarithm for data anlaysis.

[def]Means in the same column for the same element with different superscripts differ (P<.01).

Table 34. The concentration of minerals in 0, 1, 2, 3 week milk of sows fed 5000 ppm added Zn

		Cu			Fe		
Time(wk)	N	Mean µg/ml	Transformed mean[a]	Se	Mean µg/ml	Transformed mean[a]	SE
0	15	.40	1.52[d]	.10	2.28	3.14	.09
1	11	.24	1.02[e]	.11	2.29	3.12	.10
2	9	.28	1.27[de]	.13	2.12	3.07	.11
3	9	.21	1.03[e]	.13	2.67	3.22	.11
		Zn			Ca		
	N	Mean µg/ml	Transformed mean[a]	SE	Mean %	Transformed mean[b]	SE
0	15	19.35	5.13[d]	.09	.07	2.01[d]	.05
1	11	10.27	4.62[e]	.10	.20	3.02[e]	.06
2	9	10.66	4.65[e]	.11	.24	3.18[e]	.07
3	9	10.81	4.66[e]	.11	.24	3.21[e]	.07
		Mg			P		
	N	Mean µg/ml	Transformed mean[c]	SE	Mean %	Transformed mean[b]	SE
0	15	101.80	4.65[d]	.06	.12	2.40[d]	.06
1	11	128.08	4.84[de]	.07	.15	2.74[e]	.07
2	9	134.80	4.89[e]	.08	.15	2.78[e]	.08
3	9	142.36	4.96	.09	.16	2.84[e]	.08

[a]Individual values were multiplied by 10, had 1 added and were then transformed to their natural logarithm for data analysis.

[b]Individual values were multiplied by 100, had 1 added and were then transformed to their natural logarithm for data analysis.

[c]Individual values had 1 added and were transformed to their natural logarithm for data anlaysis.

[de]Means in the same column for the same element with different superscripts differ ($P<.01$).

stage of lactation. Magnesium in the colostrum of sows fed 5000 ppm added zinc was lower than at 14 and 21 days post-farrowing. This dietary treatment affected copper in the milk more than the other treatments.

Miller et al. (1965) found that supplementing lactating dairy cows with 0, 500, 1000 and 2000, ppm zinc as zinc oxide resulted in the average milk zinc levels of 4.1, 6.7, 8.0 and 8.4 ppm, respectively. Thus, the added increments of dietary zinc had progressively less effect, so that those given 1000 ppm had essentially the same amount of zinc in their milk as cows receiving 2000 ppm dietary zinc. Milk production was not affected in this 6 week trial. Because feeder cattle have been reported to have reduced gains and lowered feed efficiency (Ott, et al., 1966c) when fed 900 ppm zinc, reduced milk production might have been expected. In this study, the zinc concentration in the milk of gilts and sows fed 5000 ppm additional zinc was significantly higher at 7 and 14 days of lactation (table 28) but progressively increasing zinc levels in milk was not observed when the lower levels of zinc were consumed. As in this study, Miller et al. (1965) did not see an effect on magnesium in cow's milk from 1000 or 2000 ppm supplemental zinc, but Hamilton et al. (1979) observed depressed magnesium concentrations in the duodenum and liver of Japanese quail fed zinc at levels of 250, 500, 1000 and 2000 ppm. The iron concentration was also depressed in the same organs. Magee and Matrone (1960) observed a decrease in iron liver stores in rats fed high levels of zinc from either zinc chloride, zinc carbonate or zinc oxide. Thus, it would appear that some of the mineral interrelationships observed in other body tissues exist in mammary secretions.

Comparison of parity effects within dietary treatment. Although total milk production in sows is believed to maximize at three to four years of age, the effect of parity on mineral concentration in the milk of sows does not appear to have been investigated. When sows were fed 0 ppm added zinc, the iron concentration in the milk (table 35) was depressed in the second parity at 7 and 14 days of lactation. The phosphorus content was depressed at 21 days in the second parity, but calcium was increased at 7 and 14 days in this later parity.

It is difficult to assess the significance of these results since calcium, phosphorus and iron concentrations in the milk are believed to be immune to dietary effects (Underwood, 1977) but appear to be affected by parity with no added zinc in the diet.

When 50 ppm of zinc was added to the diet, iron and zinc were depressed in 7 day milk of second parity sows, and calcium was increased in 14 day milk (table 36) in the later parity. Phosphorus was lower ($P<.05$) in the second parity milk in all time periods except 2 weeks and magnesium was decreased in the second parity colostrum. Since, NRC (1979) recommends 50 ppm in dietary zinc for sows, this treatment represents the pattern of mineral concentration in milk that would be observed in many sows.

Sows receiving 500 ppm added zinc in their diets produced milk with less copper in the colostrum by second parity (table 37). Iron and magnesium were depressed in second parity milk at all time periods except 21 days, and phosphorus was depressed in second parity milk collected at 7 days of lactation.

At our highest level of zinc supplementation (5000 ppm) parity affected iron, zinc, magnesium and phosphorus at certain time periods

Table 35. Concentration of minerals in first and second parity milk from sows fed 0 ppm added zinc

Time(wk)	Parity 1				Parity 2			
Copper	N	Mean µg/ml	Transformed mean[a]	SE	N	Mean µg/ml	Transformed mean[a]	SE
0	11	3.93	3.63	.04	9	3.71	3.46	.04
1	11	1.61	2.79	.04	9	1.40	2.70	.04
2	11	1.32	2.64	.04	9	1.24	2.60	.04
3	11	1.29	2.61	.04	9	1.23	2.58	.04
Iron	N	Mean µg/ml	Transformed mean[a]	SE	N	Mean µg/ml	Transformed mean[a]	SE
0	11	2.21	3.11	.07	9	2.11	3.07	.08
1	11	2.66	3.22[a]	.07	9	1.53	2.76[b]	.08
2	11	2.27	3.14[a]	.07	9	1.82	2.93[b]	.08
3	11	1.86	2.92	.07	9	1.74	2.89	.08
Zinc	N	Mean µg/ml	Transformed mean[a]	SE	N	Mean µg/ml	Transformed mean[a]	SE
0	11	13.22	4.86	.10	9	16.67	5.08	.11
1	11	6.61	4.18	.10	9	6.41	4.15	.11
2	11	7.31	4.29	.10	9	6.19	4.13	.1
3	11	6.56	4.19	.10	9	6.56	4.18	.11
Calcium	N	Mean %	Transformed mean[b]	SE	N	Mean %	Transformed mean[b]	SE
0	11	.07	1.99	.05	9	.07	2.08	.06
1	11	.17	2.85[a]	.05	9	.21	3.09[b]	.06
2	11	.19	2.98[a]	.05	9	.22	3.14[b]	.06
3	11	.20	3.05	.05	9	.22	3.14	.06
Magnesium	N	Mean µg/ml	Transformed mean[c]	SE	N	Mean µg/ml	Transformed mean[c]	SE
0	11	99.6	4.61	.05	9	96.6	4.54	.05
1	11	126.4	4.83	.05	9	119.8	4.79	.05
2	11	128.7	4.86	.05	9	128.1	4.85	.05
3	11	130.9	4.87	.05	9	130.2	4.87	.05
Phosphorus	N	Mean %	Transformed mean[b]	SE	N	Mean %	Transformed mean[b]	SE
0	11	.13	2.66	.05	9	.11	2.48	.05
1	11	.15	2.79	.05	9	.13	2.64	.05
2	11	.16	2.85	.05	9	.14	2.71	.05
3	11	.16	2.80[a]	.05	9	.13	2.61[b]	.05

[a]Individual values were multiplied by 10, had 1 added and then transformed to their natural logarithm for data analysis.

[b]Individual values were multiplied by 100, had 1 added and then transformed to their natural logarithm for data analysis.

[c]Individual values had 1 added and were transformed to their natural logarithm for data anlaysis.

[de]Means on the same line with different superscripts differ (P<.05).

Table 36. Concentration of minerals in first and second parity milk from sows fed 50 ppm added zinc

Time(wk)	Parity 1				Parity 2			
Copper	N	Mean µg/ml	Transformed mean[a]	SE	N	Mean µg/ml	Transformed mean[a]	SE
0	13	2.86	3.26	.04	10	2.56	3.17	.04
1	13	1.39	2.69	.04	10	1.28	2.56	.04
2	13	1.30	2.62	.04	9	1.06	2.44	.04
3	13	1.16	2.50	.04	9	1.18	2.54	.04
Iron	N	Mean µg/ml	Transformed mean[a]	SE	N	Mean µg/ml	Transformed mean[a]	SE
0	13	2.28	3.14	.07	10	1.81	2.92	.08
1	13	2.13	3.07[d]	.07	10	1.52	2.75[e]	.08
2	13	1.45	2.71	.07	9	1.22	2.61	.08
3	13	1.79	2.91	.07	9	1.76	2.90	.08
Zinc	N	Mean µg/ml	Transformed mean[a]	SE	N	Mean µg/ml	Transformed mean[a]	SE
0	13	15.98	4.95	.10	10	12.81	4.78	.10
1	13	6.88	4.23[d]	.10	10	4.81	3.78[e]	.10
2	13	6.52	4.17	.10	9	6.29	4.15	.11
3	13	7.21	4.28	.10	9	7.74	4.36	.11
Calcium	N	Mean %	Transformed mean[b]	SE	N	Mean %	Transformed mean[b]	SE
0	13	.09	2.28	.05	10	.09	2.23	.05
1	13	.18	2.94	.05	10	.21	3.10	.05
2	13	.18	2.94[d]	.05	9	.22	3.13[e]	.06
3	13	.22	3.13	.05	9	.22	3.15	.06
Magnesium	N	Mean µg/ml	Transformed mean[c]	SE	N	Mean µg/ml	Transformed mean[c]	SE
0	13	120.8	4.78[d]	.04	10	84.2	4.42[e]	.05
1	13	123.8	4.82	.04	10	113.1	4.71	.05
2	13	125.6	4.84	.04	9	114.8	4.74	.05
3	13	133.2	4.90	.04	9	118.8	4.77	.05
Phosphorus	N	Mean %	Transformed mean[b]	SE	N	Mean %	Transformed mean[b]	SE
0	13	.14	2.73[d]	.04	10	.12	2.55[e]	.05
1	13	.15	2.79[d]	.04	10	.12	2.57[e]	.05
2	13	.14	2.73	.04	9	.13	2.62	.05
3	13	.17	2.86[d]	.04	9	.13	2.60[e]	.05

[a]Individual values were multiplied by 10, had 1 added and were then transformed to their natural logarithm for data analysis.
[b]Individual values were multiplied by 100, had 1 added and were then transformed to their natural logarithm for data analysis.
[c]Individual values had 1 added and were transformed to their natural logarithm for data anlaysis.
[de]Means on the same line with different superscripts differ (P<.05).

Table 37. Concentration of minerals in first and second parity milk from sows fed 500 ppm added zinc

Time(wk)	Parity 1				Parity 2			
Copper	N	Mean µg/ml	Transformed mean[a]	SE	N	Mean µg/ml	Transformed mean[a]	SE
0	12	3.48	3.45[d]	.04	10	2.51	3.11[e]	.04
1	12	1.38	2.67	.04	10	1.54	2.79	.04
2	12	1.18	2.53	.04	9	1.10	2.47	.04
3	9	1.10	2.46	.04	8	1.08	2.46	.05
Iron	N	Mean µg/ml	Transformed mean[a]	SE	N	Mean µg/ml	Transformed mean[a]	SE
0	12	2.22	3.11[d]	.07	10	1.75	2.86[e]	.08
1	12	2.19	3.11[d]	.07	10	1.48	2.72[e]	.08
2	12	1.99	3.02[d]	.07	9	1.611	2.78[d]	.08
3	12	1.73	2.84	.07	8	1.48	2.66[f]	.09
Zinc	N	Mean µg/ml	Transformed mean[a]	SE	N	Mean µg/ml	Transformed mean[a]	SE
0	12	15.23	4.95	.10	10	12.32	4.73	.10
1	12	6.91	4.22	.10	10	6.62	4.17	.10
2	12	7.22	4.27	.10	9	5.80	4.06	.11
3	12	7.89	4.37	.10	8	7.68	4.33	.12
Calcium	N	Mean %	Transformed mean[b]	SE	N	Mean %	Transformed mean[b]	SE
0	12	.08	2.23	.05	10	.08	2.22	.05
1	12	.18	2.96	.05	10	.19	2.95	.05
2	12	.19	3.00	.05	9	.21	3.06	.06
3	12	.21	3.10	.05	8	.24	3.19	.06
Magnesium	N	Mean %	Transformed mean[c]	SE	N	Mean %	Transformed mean[c]	SE
0	12	116.3	4.76[d]	.05	10	86.1	4.45[e]	.05
1	12	124.1	4.83[d]	.05	10	104.3	4.64[e]	.05
2	12	134.2	4.89[d]	.05	9	105.8	4.65[e]	.05
3	12	134.7	4.91	.05	8	135.4	4.90	.06
Phosphorus	N	Mean %	Transformed mean[b]	SE	N	Mean %	Transformed mean[b]	SE
0	12	.13	2.65	.05	10	.12	2.55	.05
1	12	.16	2.82[d]	.05	10	.12	2.57	.05
2	12	.16	2.82	.05	9	.13	2.66	.05
3	12	.16	2.83	.05	8	.15	2.76	.06

[a]Individual values were multiplied by 10, had 1 added and were then transformed to their natural logarithm for data analysis.

[b]Individual values were multiplied by 100, had 1 added and were then transformed to their natural logarithm for data analysis.

[c]Individual values had one added and were transformed to their natural logarithm for data anlaysis.

[de]Means on the same line with different superscripts differ (P<.05).

(table 38). Iron was depressed at 7 days in the second parity; zinc was decreased at 7 and 14 days of lactation in the second parity and magnesium and phosphorus were reduced in colostrum in this later stage of lactation.

When evaluating these results it should be remembered that there is isotopic evidence that all minerals in milk must come from the plasma (Linzell, 1968), yet one would not expect the age associated with an additional parity to affect these parameters. Miller (1965) suggested that the udder was able to discriminate against zinc at high dietary levels. Perhaps there is an active transport mechanism for controlling the concentration of minerals in milk. If not, mineral concentrations in milk should mirror that of plasma. Henkin et al. (1970) suggested that prolactin was positively correlated with copper and zinc in milk of rats and humans. Since iron was lower in the second parity milk at 7 days of lactation in all treatments and magnesium was decreased in the second parity colostrum in all but one treatment, it suggests that prolactin or some hormone may be affecting the minerals in milk. Additional work utilizing endocrine techniques is needed to elucidate the control of mineral concentrations in milk.

Table 38. Concentration of minerals in first and second parity milk from sows fed 5000 ppm added zinc

Time(wk)	Parity 1				Parity 2			
Copper	N	Mean µg/ml	Transformed mean[a]	SE	N	Mean µg/ml	Transformed mean[a]	SE
0	9	.41	1.51	.04	6	.38	1.53	.05
1	7	.23	0.97	.05	5	.25	1.10	.06
2	5	.28	1.25	.06	4	.28	1.30	.07
3	5	.20	1.21	.06	4	.23	1.07	.07
Iron	N	Mean µg/ml	Transformed mean[a]	SE	N	Mean µg/ml	Transformed mean[a]	SE
0	9	2.48	3.23	.08	6	19.8	3.00	.10
1	7	3.16	3.40[d]	.09	4	1.35	2.64[e]	.12
2	5	2.10	3.06	.11	4	2.15	3.08	.12
3	5	2.64	3.23	.11	4	2.70	3.21	.12
Zinc	N	Mean µg/ml	Transformed mean[a]	SE	N	Mean µg/ml	Transformed mean[a]	SE
0	9	21.19	5.31[d]	.11	6	16.60	4.86[e]	.14
1	7	10.99	4.69[de]	.13	4	9.00	4.49	.17
2	5	11.22	4.72	.15	4	9.95	4.57	.17
3	5	11.20	4.69	.15	4	10.33	4.60	.7
Calcium	N	Mean %	Transformed mean[b]	SE	N	Mean %	Transformed mean[b]	SE
0	9	.07	2.01	.06	6	.07	2.02	.07
1	7	.19	2.99	.06	4	.21	3.07	.09
2	5	.21	3.07	.08	4	.27	3.31	.09
3	5	.21	3.10	.08	4	.28	3.36	.09
Magnesium	N	Mean µg/ml	Transformed mean[c]	SE	N	Mean µg/ml	Transformed mean[c]	SE
0	9	118.6	4.77[d]	.05	6	76.5	4.46[e]	.06
1	7	131.1	4.87	.06	4	122.8	4.80	.08
2	5	128.8	4.86	.07	4	142.3	4.92	.08
3	5	132.4	4.89	.07	4	154.8	5.05	.08
Phosphorus	N	Mean %	Transformed mean[b]	SE	N	Mean %	Transformed mean[b]	SE
0	9	.12	2.55[d]	.05	6	.11	2.16[e]	.06
1	7	.16	2.83	.06	4	.13	2.60	.08
2	5	.16	2.85	.07	4	.14	2.68	.08
3	5	.16	2.84	.07	4	.16	2.85	.08

[a]Individual values were multiplied by 10, had 1 added and were then transformed to their natural logarithm for data analysis.
[b]Individual values were multiplied by 100, had 1 added and were then transformed to their natural logarithm for data analysis.
[c]Individual values had 1 added and were transformed to their natural logarithm for data anlaysis.
[de]Means on the same line with different superscripts differ ($P<.05$).

APPENDIX

APPENDIX

Table 39. Effect of 0, 5 or 10 ppm dietary copper on weight and blood parameters

	Diet Cu, ppm		
Weight, kg	0	5	10
0 days	2.62	2.69	2.47
14 days	4.81[a]	6.56[b]	6.01[ab]
35 days	8.40[a]	16.90[b]	15.54[b]
Serum Cu, µg/dl			
0 days	12	11	9
7 days	6[a]	97[b]	108[b]
35 days	11[a]	213[b]	190[b]
Hemoglobin, g/dl			
0 days	8.9	9.6	9.1
7 days	7.1[a]	10.7[b]	10.3[b]
35 days	5.5[a]	12.2[b]	12.0[b]
Hematocrit, %			
0 days	28.6	30.5	28.4
7 days	23.2[a]	32.8[b]	32.7[b]
35 days	21.2[a]	37.9[b]	37.9[b]

[ab]Means on the same line with different superscripts differ significantly ($P<.05$).

Table 40. Effect of 0, 5 or 10 ppm dietary copper on enzymes

	Diet Cu, ppm		
	0	5	10
Ceruloplasmin, ΔOD/min/ml			
0 days	.004	.005	.008
7 days	.000[a]	.246[b]	.207[b]
35 days	.000[a]	.499[b]	.474[b]
Aortic lysyl oxidase activity, dpm/μg extracted protein			
Insoluble elastin as substrate	7.2[a]	19.9[b]	14.8[ab]
Soluble elastin as substrate	16.9[a]	118.6[b]	93.3[b]
Soluble collagen as substrate	2.0[a]	5.2[b]	4.0[b]
Aortic lysyl oxidase activity, dpm/mg tissue			
Insoluble elastin as substrate	78.2	62.4	63.8
Soluble elastin as substrate	12.9[a]	418.2[b]	330.9[b]
Soluble collagen as substrate	11.2	16.1	15.1
Cytochrome C oxidase activity, ΔOD/min/mg protein			
Heart tissue	.53[a]	3.81[b]	3.72[b]
Liver tissue	.35[a]	.91[b]	.95[b]

[ab]Means on the same line with different superscripts differ significantly ($P<.05$).

Table 41. Effect of 0, 5 or 10 ppm dietary copper on relative organ weights and mineral concentrations in tissues

	Diet Cu, ppm		
	0	5	10
Zn concentration in tissues, ppm, wet basis			
Liver	73[a]	127[b]	103[ab]
Heart	8[a]	11[ab]	12[b]
Kidney	14[a]	19[b]	15[a]
Pancreas	35	45	40
Spleen	13	14	14
Muscle	14	18	12
Hair	167[a]	146[ab]	142[b]
Rib bone (9 and 10)	218[a]	138[b]	154[b]
Cu concentration in tissues, ppm, wet basis			
Liver	.28[a]	15.90[b]	15.88[b]
Heart	.17[a]	2.59[b]	2.48[b]
Kidney	1.95[a]	5.31[b]	3.98[ab]
Pancreas	.33[a]	1.07[b]	1.21[c]
Spleen	.62	.96	.97
Muscle	.09[a]	.55[b]	.41[c]
Hair	3.03[a]	7.40[b]	8.05[b]
Fe in tissues, ppm, wet basis			
Liver	52.0[a]	23.4[b]	25.0[b]
Hair	10.67[a]	7.40[b]	7.50[b]
Ca and P in rib bone (9 and 10), %			
Ca	12.87	16.50	17.67
P	5.70	6.48	6.10
Relative organ wt.			
Heart	1.13[a]	.46[b]	.48[b]
Liver	3.10	2.55	2.55
Kidney	.67[a]	.51[b]	.52[b]
Spleen[c]	.21	.15	.17
Pancreas	.17	.18	.17
Adrenal glands	.02[a]	.01[b]	.01[b]
Thyroid gland[c]	.009	.010	.013

[ab]Means on the same line with different superscripts differ significantly ($P<.05$).
[c]0 ppm vs 5 and 10 ppm significantly different.

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