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I am submitting herewith a thesis written by John Rocco Moore entitled "Effect of Nickel Concentration, Iron Concentration, and Iron Form on Performance, Hematological, Tissue and Mineral Parameters of Young Poultry and Weaned Pigs." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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EFFECT OF NICKEL CONCENTRATION, IRON CONCENTRATION, AND IRON FORM ON PERFORMANCE, HEMATOLOGICAL, TISSUE AND MINERAL PARAMETERS OF YOUNG POULTRY AND WEANED PIGS

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ABSTRACT

Possible physiological interactions between nickel and iron were investigated in chicks and weaned baby pigs with special emphasis on the effect of form and concentration of iron on nickel and their effects on performance, hematology and mineral parameters.

In the first experiment 48 Yorkshire X Duroc X Hampshire weaned pigs from 8 litters and averaging 9.8 kg were housed individually in 76 X 60 cm expanded metal pens. They were divided into 8 groups and fed diets containing 0, 10, 50 or 100 parts per million (ppm) added nickel (Ni) as the chloride and 60 ppm iron (Fe) as ferrous or ferric sulfate in a 4 X 2 factorial arrangement. Feed mixed with water as a gruel was offered twice daily to appetite and additional water was to satiation after each feeding. Pigs were weighed and blood was sampled weekly to obtain performance, hematological data and plasma minerals (Ni, Fe, Cu and Zn). Hair was clipped from the right shoulder and hip at 14, 21 and 28 days for mineral analysis.

Pigs fed Ni added at 50 ppm to the basal diet had lower ($P<.05$) average daily gain (ADG) after 14 days and required more ($P<.05$) feed per unit gain (F/G) than did pigs fed the other Ni concentrations. Pigs receiving ferric Fe also required more F/G than did those given ferrous Fe. Although not affected significantly by form of Fe across all concentrations of Ni, ADG was decreased with ferric but not with ferrous Fe at 50 ppm added Ni. Addition of 50 ppm Ni increased ($P<.05$) red cell concentration, hematocrit and hemoglobin at 28 days and concentrations of Ni, Fe and Cu in pigs fed 50 ppm added Ni were higher when ferric as compared with ferrous Fe was fed. Dietary

concentration of nickel and form of Fe had no effect on mineral content of hair.

Addition of 50 ppm Ni decreased performance and increased hematological and plasma minerals in pigs. Nickel chloride appeared to interact in a synergistic manner with ferric sulfate but not with ferrous sulfate on the parameters studied.

In the second experiment 384 male broiler chicks of the Hubbard X Hubbard strain were fasted overnight, weighed to the nearest gram, wing banded and allotted to 48 treatment groups with similar average initial weights. Groups were assigned to 48 wire bottomed cages within two batteries containing 24 cages each. Three replicates per treatment with eight birds per replicate were randomly allotted to 0, 10, 50 or 100 ppm added nickel from nickel chloride, each with 60 or 100 ppm Fe as ferrous sulfate in a 4 X 2 X 2 factorial arrangement. The birds were fed and watered ad libitum for the 4 weeks of the experiment. They were weighed at the start of the experiment and thereafter at weekly intervals. At the termination of the experiment all chicks were bled via heart puncture and 12 tissues were obtained from each bird for analysis.

Average daily feed intake (ADF) and ADG were higher ($P<.05$) for chicks fed 60 ppm as compared with 100 ppm Fe for the first 2 weeks of the experiment. However, at day 21 ADG was higher ($P<.05$) when Fe was supplemented at 100 ppm rather than at 60 ppm. On day 28 ADG was higher ($P<.05$) for chicks fed diets supplemented with ferric Fe rather than for those fed ferrous Fe supplemented diets. However, ADF was significantly higher for chicks fed diets supplemented with

ferrous Fe than when ferric Fe was fed. Feed:gain ratio was not affected significantly by either form or concentration of Fe.

Hematocrit was affected by neither form nor concentration of Fe. Although not affected by concentration of Fe, hemoglobin concentration was higher ($P<.05$) when chicks were supplemented with ferric rather than ferrous Fe. Nickel concentration in trichloroacetic acid treated plasma was increased ($P<.05$) for chicks fed diets supplemented with ferrous Fe. Plasma Fe concentration was higher ($P<.05$) for chicks fed 60 rather than 100 ppm Fe and higher ($P<.05$) when ferrous rather than ferric Fe was added.

Average daily gain was increased ($P<.05$) on day 7 by Ni added at 50 and 100 ppm, was decreased ($P<.05$) on days 14 and 21 by 100 ppm added Ni, but at day 28, ADG and total ADG were not significantly affected by Ni concentration. Neither ADF nor feed:gain were significantly affected by added dietary Ni. Added Ni decreased ($P<.05$) hematocrit at 50 ppm but increased ($P<.05$) hemoglobin concentration at 100 ppm. Plasma Ni concentration was increased ($P<.05$) by added dietary Ni at 50 and 100 ppm when plasma was treated with sulfosalicyclic acid and at 100 ppm when plasma was treated with TCA. Nickel concentration was higher when plasma was treated with TCA than when plasma was treated with SSA. Concentrations (wet basis) of Ni and Fe were higher ($P<.05$) in kidney tissue in chicks fed 100 ppm added Ni.

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CHAPTER I

INTRODUCTION

Nickel is a white ferromagnetic metal that occurs in great abundance throughout nature. Nickel is one of the major components of the earth with an average concentration of approximately 2% by weight, making it the fifth most abundant element. Along with iron, whose biological essentiality was known as early as the 17th century (Schwarz, 1977), and cobalt, which was identified as the central atom of vitamin B_{12} in the eighth subgroup of the periodic system. Its atomic weight is 58.71 and its atomic number is 28. The beta-emitting isotope of nickel is ^{63}Ni, which has a half-life of 92 years. The preferred oxidation states of nickel are 0 and +2. Nickel reacts with carbon monoxide and hydrogen to form nickel tetracarbonyl ($Ni(CO_2)_4$) and reactive nickel hydride (NiH_2).

Because of the chemical and physical relationships between nickel, iron, and cobalt, a number of attempts were made to demonstrate the physiological essentiality of nickel in the 1950's and 1960's. These experiments on diet supplementation of nickel were conducted utilizing rats (Schroeder et al., 1962) and, although comprehensive, they did not produce significant results at the concentration tested because the nickel content of the rations was sufficient to meet all nickel requirements.

Recently a great deal of interest has developed in the essentiality, metabolism, and toxicity of nickel. Since 1975 newer and more accurate methods of analysis of trace elements have become

available to the trace element researcher. This has been particularly beneficial with elements such as nickel that are generally found in relatively small quantities in physiological fluids and tissues.

Nickel deficiencies have been produced in pigs, chicks, rats, cattle, horses, and goats. When animals showing nickel deficiency symptoms were fed supplemental concentration of nickel, the deficiency symptoms were elevated. Nielsen and Ollerich (1974) obtained evidence of nickel deficiencies in chicks. Long term studies have demonstrated certain specific long term effects from low concentration nickel deficiency, including reproductive inefficiency and increased natal mortality (Schroeder et al., 1974).

Nickel has been shown to interact with a number of other minerals, specifically iron, copper, and zinc (Nielsen and Zimmerman, 1981; Spears et al., 1978a).

Nickel has been reported to decrease iron absorption and when fed at high concentrations causes iron deficiency symptoms when the dietary iron concentrations are adequate to meet the normal needs of the animal (Nielsen and Zimmerman, 1981). Nickel absorption was increased under conditions of iron deficiencies, suggesting a common metabolic pathway.

Nickel is relatively nontoxic when viewed in terms of the amount required, above normal concentrations found in most rations, to cause toxic symptoms. It appears that diet composition, protein concentrations, age, and nutritional status of the animal have a great influence on the concentrations of dietary nickel necessary to

cause toxicosis. Most tissues contain measurable concentrations of nickel, with the heart, bone, kidney, liver, blood, and spleen containing the highest concentrations of nickel (Phatak and Patwardhan, 1952).

Nickel's essentiality has been well documented in the diet of many animals, but the metabolic mechanisms for essential functions of nickel are not well defined at this time. Therefore, this study was undertaken to more clearly define the possible physiological interaction between nickel and iron with special emphasis on the effect of form and concentration of iron on nickel and their effects on performance, hematological and mineral parameters of weaned pigs and young chicks.

CHAPTER II

LITERATURE REVIEW

I. General Considerations

Nickel (Ni) is a hard silver white ferromagnetic metal that can be polished to a high lustrous finish. It occurs in nature in several ores (chalcopyrite, pyrrhotite, pentlandite, garnierite, and niccolite) and comprises approximately 0.008% of the earth's crust. Nickel has several industrial uses including use in alloys, storage batteries, electroplating and as a catalyst used for hydrogenation of organic compounds (National Research Council, 1975).

During recent years considerable interest has developed in the essentiality, metabolism, toxicity, and interactions of nickel with other minerals.

The hypothesis that nickel plays an important role in nonruminant nutrition is based primarily upon the following considerations. The position of nickel in the periodic table in relation to other more clearly defined essential elements, the binding of nickel to alpha-globulin in the blood plasma of some animals, the occurrence of nickel in the tissues of animals, the in vitro activation of some enzymes by nickel, and the essentiality of nickel for rats, chickens, cattle, and goats in recent nutritional studies.

When diets that are very low in nickel are fed to animals maintained in exceptionally clean environments, a marked decrease in normal growth, development and reproduction was evident (National

Research Council, 1980). Diets containing 20 ppb or less nickel resulted in the occurrence of several abnormalities which could be reversed by the addition of nickel to the diet (Ling and Leach, 1979).

The biological effects of most nutrients are generally studied alone with very little regard for possible interacting effects with other nutrients. This approach is possibly due to the fact that most scientists are specialists who prefer to design and conduct experiments that are unifactorial rather than multifactorial. In reality animals are constantly exposed to multiple nutritional situations, so that multifactorial studies are generally justified.

II. Essentiality

Nielsen and Ollerich (1974) reported the first evidence of nickel deficiencies in chicks. Diets used to produce nickel deficiencies were very low in dietary nickel with a range of between 2 and 40 ppb nickel. When studying deficiency and essentiality of nickel it was necessary to maintain all experimental animals in filtered air environments with nickel sources very rigorously excluded. It was also necessary to feed the low nickel diets throughout a lifetime or through more than one generation. The requirement was estimated to be between 50 and 80 ppb for the rat and chick (Nielsen and Sandstead, 1974).

Nickel deficiencies have been produced in pigs, chicks, and rats, by (Nielsen and Sandstead, 1974), and in sheep (Spears and Hatfield, 1978). Abnormalities that have been observed in deficient

animals varied among species, laboratories, and within laboratories, on successive experiments. The differences were attributable to the degree of the deficiency, diet composition, inadequacies in the filtered environment, and the adequacy of the diet in nutrients other than nickel. The problems with dietary adequacy and environmental control in the duplication of deficiency symptoms have been greatly improved for chicks (Nielsen et al., 1975a) and rats (Nielsen et al., 1975b). The abnormalities observed in chicks that were nickel deficient included less yellow lipochrome pigment in the shank skin, abnormalities in the rough endoplasmic reticulum of the liver, and depressed hematocrits (Nielsen et al., 1975a). A very similar liver pathology has been observed in nickel deficient chicks by Sunderman et al., (1972a). Delayed sexual maturity was observed in the sow, and a high mortality rate has been shown in young pigs with a decreased growth rate, impaired reproduction, and rough hair coat on a 100 ppb nickel supplemented diet fed to swine (Sunderman et al., 1972a).

Nielsen et al., (1975b) reported that rats raised through successive generations on nickel deficient diets resulted in unthriftiness and uneven hair development in pups, increased neonatal mortality, decreased liver cholesterol and ultrastructural changes in the liver.

In a study by Anke et al., (1974) nickel deficiency was produced in miniature swine that were fed a diet containing 10 ppm nickel. Approximately 20 to 30% of the pigs on the nickel deficient diet developed a scaly and crusty skin, which was very similar to

parakeratosis, during the preweaning period. Pigs which were deficient in nickel also had lower concentrations of Zn in hair, rib, liver, and brain tissue. Chung et al., (1976) reported that nickel supplementation at 54 ppm increased gain and feed efficiency in growing finishing swine fed diets low in zinc. Incidence of parakeratosis was reduced by the addition of nickel. Spears et al., (1984) placed day-old pigs on a low nickel liquid diet (0.16 ppm) supplemented with either 0.5 or 25 ppm nickel, on a dry matter basis, for a 21 day period. Dietary nickel did not effect animal gain, liver cholesterol, serum protein concentrations or bacterial urease activity in the gastrointestinal tract.

Kirchgessner and Schnegg (1980) determined a nickel requirement of 0.7 mg Ni/kg ration on a dry matter basis from balance experiments with pregnant and nonpregnant sows, but their results may have been affected by the retention of nickel during pregnancy.

III. Metabolism

Nickel deficiency has been developed in five different animal species, but a specific metabolic role for nickel in the mammalian or avian system has not been identified. A number of functions and effects on metabolic processes have been proposed based on in vitro and in vivo research results (Spears, 1984).

Fishbein et al., (1976) were among the first to show the existence of a metalloenzyme containing nickel. The enzyme was urease which was extracted from the jackbean. The jackbean urease was found to contain 6 to 8 atoms of nickel per mole of the enzyme

with a molecular weight of 480,000 per mole of enzyme. It has also been shown that Ni, Mn, and Cd were inhibitory to ruminal bacteral urease (Spears and Hatfield, 1978).

Sunderman (1965) demonstrated that nickel tends to be localized in the nucleic acid fraction of the nucleus. Sunderman (1965) also reported that low nickel diets caused a marked decrease in Kreb's Cycle activity and electron transport ability. Cytochrome oxidase, isocitrate dehydrogenase, and malic dehydrogenase are the primary enzymes affected (Weber and Reid, 1969). Sunderman et al., (1972b) isolated a nickel-metalloprotein called "nickeloplasmin" from rabbit and human serum. These findings are consistent with other findings which suggest that nickel has a role in nucleic acid and/or protein metabolism. Webster (1965) found that nickel was needed for the formation of an enzyme bound acetylodenylate, an intermediate, necessary for the formation of acetyl CoA from ATP, acetate, and CoA.

Spears et al., (1978a) reported that protein digestion in ruminants may be affected by nickel concentrations in the diet. Urinary nitrogen was lower and the percentage of absorbed nitrogen was greater for lambs fed supplemental nickel. Weber and Reid (1969) reported that the digestibility of protein, energy, fat, and bone metabolism were unaffected by dietary nickel.

Nielsen et al., (1975b) suggested that nickel may play a role in the structure and function of membranes as evident by ultrastructural changes during nickel deficiency.

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Nickel can replace many of the naturally occurring metals in the metalloenzymes, and the resulting enzyme activities have been found to be very similar to those found in natural metalloenzymes.

Coleman and Vallee (1961) demonstrated that nickel can replace the natural metals in carboxypeptidases, while Ray and Maltani (1972) observed that nickel could replace cobalt, the central metal, in phosphoglucomutase.

Unabsorbed nickel in the gastrointestinal tract is lost in feces while absorbed nickel appears to be primarily excreted by the urinary tract in most animals and man.

Hair has been studied by several laboratories as a means of rapid noninvasive measurement of intestinal absorption relationships. This method of measurement of internal exposure has been complicated by external contamination of the hair surface and nonstandardized cleaning methods, as well as differences in sampling techniques. The conflicting results obtained are probably as a direct result of these factors (National Research Council, 1975).

A number of diseases and physiological stresses are known to influence the in vivo kinetics of nickel metabolism. It has been demonstrated that in man, serum nickel concentrations increase in response to acute myocardial infarction (Sunderman et al., 1972b). McNeely et al., (1971) reported an increase in serum nickel concentrations in extensive burn injury in man. Serum nickel concentrations are decreased in cases of hepatic cirrhosis and uremia as well as in individuals exposed to extremes of heat (National Research Council, 1975). Kohlhardt et al., (1979) reported a

significant contractile activity depression after the injection of higher Ni doses (2-20 mg/kg $NiCl_2$) in studies with isolated ventricular myocardium. In a study by Ligeti (1980) exogenously administered $NiCl_2$ caused a significant decrease of coronary vascular conductance without a change in the total serum Ni concentrations, hemodynamic or cardiac mechanical parameters of the anesthetized dog. Higher Ni doses caused a further reduction of coronary blood flow, an increase in serum Ni concentration and a depression of heart rate and ventricular contractility. Experimental results demonstrated that trace amounts of Ni reduced coronary blood flow by a local action on coronary vessels.

As a result of these types of responses to various physiological stresses, one must exercise great care in interpreting serum and tissue concentrations of nickel in animals used for nutrition research.

No mammalian enzyme has been shown to require nickel as its primary component, and no enzyme has been shown to by activated by nickel alone (Spears, 1984). Schnegg and Kirchgessner (1977) reported a definite liver enzyme effect from nickel deficiency in 30-day old rats. No impairment of the liver enzymes by a reduced absorption of the dietary protein was demonstrated, nor was total protease activity diminished by nickel depletion. Schnegg and Kirchgessner (1977) also investigated the effect of nickel depletion on the hepatic concentration of various metabolites and found no significant effects on hepatic concentrations of these metabolites.

Rabbit serum contained three nickel-binding fractions: (1) ultrafiltrable nickel (16%); (2) 2-macro-globulin bound nickel named nickeloplasmin (44%); and (3) an albumin-bound nickel (40%) (Nomoto et al., 1971). Nomoto et al., (1971) suggested that the serum ultrafiltrate constituted nickel-1-aspartate complex. These findings were in contrast to later work by Lucassen and Sarkar (1979) who proved conclusively that serum nickel was bound to L-histidine in human serum albumen.

Nickel may act to inhibit or to stimulate the release of various hormones. Nielsen (1972) reported an effect of dietary nickel on the response of chicks to epinephrine and thyroid hormone. Intraperitoneal injections of nickel, as nickel chloride, in the rat has resulted in decrease plasma insulin (Clary, 1975). Plasma glucagon has been increased by intraperitoneal injections of nickel chloride (Horak and Sunderman, 1975).

In animal nutrition, only a few situations of nickel deficiency are likely to occur under practical conditions, except where interactions with other dietary components impair absorption and metabolic efficiency of utilization of nickel (Schnegg and Kiechgessner, 1977).

IV. Sources of Nickel

Very little data are available on the nickel content of various animal feeds. Whole oats and rye seeds contain 2-3 ppm nickel, wheat ranged from 0 to 0.5 ppm, commercial dog and rat diets contained 2.1 and 3.3 ppm nickel, respectively (Schroeder et al., 1962). O'Dell et

al., (1971) reported the nickel concentrations for corn to be 0.4 ppm, oats, 1 ppm; soybean meal, 3.6 ppm; alfalfa meal, 1.4 ppm; and cotton seed hulls, 0.6 ppm.

Plant foods are generally higher in nickel than foods of animal origin, but this can vary widely depending upon the concentration of nickel in the soil in which the plants are grown.

Underwood (1977) reported that pasture plants averaged between 3.5 to 0.5 ppm nickel. These concentrations depended on species of plant, stage of maturity, nickel concentration of the soil, and nickel availability in the soil. Sapek and Sapek (1980) reported that nickel in forages decreased with advancing maturity. Soil nickel availability appears to be highly dependent upon soil pH, with nickel forming stable complexes with iron and manganese oxides at a neutral pH. As soil pH decreases and becomes more acid, nickel is released for plant uptake and as soil pH increases the nickel content of plants decreases.

The nickel content of most water is very low. The concentrations of nickel in the major river basins and water supplies of the United States were found to be less than 10 ppb (National Research Council, 1975).

Generally there is no need to add nickel to practical animal diets. Food machinery can contribute significant amounts of nickel to processed animal diets. Very little is known about the form of nickel in feed and its bioavailability. Nickel from industrial operations can contribute to the nickel concentration in air, soil, and water on a local concentration. There appears to be very few

problems for animals from exposure to these sources (National Research Council, 1975).

V. Interactions with other Minerals

In most research situations, minerals are looked at as a single entity. When trying to ascertain a mineral's biological significance, we should be careful to consider the numerous interactions that are possible. Frequently, a misleading representation of a specific mineral's biological role is advocated without considering interactions with other nutrients. Minerals may interact with enzymes, other minerals, food substances, and various elements and compounds that they may come into contact with.

Nickel interactions are numerous, with some more substantiated than others. Ling and Leach (1979) in a study with chicks, reported no interaction between nickel, cobalt, iron, copper, and zinc. When nickel was fed at toxic concentrations, the addition of the other four minerals either singularly, or in combination, did not negate the symptoms of nickel toxicity. The lack of interaction between nickel, cobalt, iron, copper, and zinc is in contrast to the results observed by other researchers investigating nickel at low dietary concentrations.

Nielsen (1980b) in a recent literature review indicated that nickel interacts with at least 13 minerals in animal and plant systems. The most researched interactions are those occurring with copper, zinc, and iron.

Spears et al., (1977) reported that an increase in weight gain and hematocrit was found in rats fed a nickel supplemented diet that was low in copper. No significant difference was found when a nickel supplemented diet was fed to rats on a diet with adequate concentrations of copper. An increase in growth rate, hematocrit, erythrocyte concentration, and ceruloplasmin was reported when copper was added to a low copper, high nickel diet. Nielsen and Zimmerman (1981) reported that there was an antagonistic interaction between Cu and Ni and that the interaction was influenced by concentrations of dietary iron.

Nickel deficiency in rats fed a diet containing iron at 50 ppm resulted in decreased hematocrits, hemoglobin concentrations, and erythrocyte concentrations (Schnegg and Kirchessner, 1977). When iron was added to the diet at 100 ppm some of the nickel deficiency symptoms were alleviated. Iron absorption was reduced by 73% in rats receiving 20 ppm nickel and iron absorption was reduced by 33% in rats receiving 15 ppm nickel. Nielsen and Zimmerman (1981) reported that the nickel and iron interaction was affected by the concentration and form of dietary iron. High dietary concentrations of iron have been shown to prevent nickel toxicity in the chick. Iron absorption appears to be reduced when fed to animals with high nickel concentrations in their diets, and iron deficiency anemia symptoms were observed under these conditions. Nickel absorption was increased under conditions of iron deficiencies, suggesting a common intestinal transport system (Becker et al., 1980).

Anke et al., (1974) reported that nickel deficiency decreased the tissue concentrations of zinc. Chung et al., (1976) reported that nickel had the ability to increase performance and zinc status of chicks and pigs that were fed a zinc deficient diet. However, Spears et al., (1978b) reported that nickel supplementation did not increase the growth rate of rats fed purified diets either severely (<1 ppm Zn) or marginally (4 to 8 ppm Zn) deficient in zinc.

VI. Toxicosis

Nickel is considered to be relatively nontoxic when considered in terms of the quantities required, above ordinary dietary intakes, to produce adverse effects in a moderate length of time.

Weber and Reid (1968) reported that dietary concentrations of 100 and 300 ppm nickel, in either the carbonate or acetate form, had no adverse effects when fed to chicks from hatching to 4 weeks of age. Dietary concentrations of 500 ppm nickel reduced growth.

Ambrose et al., (1976) reported that dogs that were fed diets with nickel concentrations at either 100 or 1,000 ppm exhibited no adverse effects after 2 years. When dogs were fed diets at 2,500 ppm nickel, they developed anemia, increased urine volume, and severe lung lesions. Young rats given 5 ppm nickel in their drinking water for their entire lifetime exhibited no adverse effects, but when the rats were continued through 3 generations, a higher infant mortality was observed in each succeeding generation of young (Schroeder and Mitchener, 1971). Schnegg and Kirchgessner (1977) reported that rats fed 1,000 ppm nickel, as the chloride, in their diets rapidly

decreased their food intake and lost weight within 2 weeks of starting the diet. There were increases in red blood cell concentration, hematocrit, hemoglobin, serum protein, nitrogen in tissues, nickel, copper, zinc and iron concentrations in some tissues. Ambrose et al., (1976) reported changes in rats that were fed 1,000 ppm nickel for 2 years. Females had reduced heart, liver, and body weight. Males exhibited no adverse effects while on the same diet.

Kirchessner and Roth (1977) reported on a study where nickel was observed to have had a toxic effect on pigs. In the study young pigs were nickel supplemented at a concentration of 375 ppm as nickel sulfate. A slight depression in gain (5%) and feed intake (3%) was observed. A nickel supplementation of 500 ppm significantly reduced gain (8%) and feed intake (6%).

O'Dell et al., (1970) found nickel chloride to be more toxic than nickel carbonate when fed at concentrations ranging form 50 to 1,000 ppm. It was concluded that nickel as the chloride was approximately 5 times more toxic than nickel as the carbonate.

It is possible that diet composition may have an important effect on dietary nickel toxicity. Lower protein concentrations in the diet have been associated with lower concentrations of nickel in animal tissues. The effect of dietary protein is an interesting avenue for future research, since nickel has been shown to reduce nitrogen retention (Weber and Reid, 1968).

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VII. Tissue Concentrations

Schroeder et al., (1974) found that tissues or body fluids with the lowest nickel concentrations, in experiments conducted with rats, were bile, serum, brain, pancreas, skin, tongue, and red blood cells. The heart, kidney, lung, and liver contained relatively high concentrations of nickel, with spleen and testes concentrations varying from moderate to high tissue nickel concentrations. Phatak and Patwardhan (1952) reported that tissue nickel concentrations of rats that were fed a diet containing 1,000 ppm nickel had decreasing concentrations of nickel in bone, heart, kidney, spleen, testes, blood, and liver, respectively.

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CHAPTER III

EXPERIMENTAL PROCEDURES

I. Experimental Animals and Facilities

Two experiments were conducted. The first experiment utilized 48 Yorkshire X Duroc X Hampshire pigs from 8 litters. The 48 crossbred pigs in experiment 1 were allotted within litter blocks by weight and with an equal number of males and females assigned to each treatment. The pigs were housed individually in expanded metal pens measuring 76 centimeters (cm) in length X 60 cm in width X 57 cm in height. Pigs were hand fed twice daily, morning and evening. Pigs were fed near ad libitum amounts at each feeding. Water was added to the diets to form a palatable gruel and after each feeding pigs were given water to satiation. The pigs were 6 to 8 weeks of age and treatment groups had averages of between 9.5 and 9.6 kilograms (kg) body weight at the initiation of the experiment. A period of 5 days was used to allow animals to acclimate to the pens, basal diets, and feeding regimen.

The second experiment utilized 384 broiler chicks of the Hubbard X Hubbard strain. The chicks were feather sexed upon hatching and only the males were saved to be allotted to experimental treatments. The chicks were allowed to become acclimated to the pens, feeding routine, and enviromental conditions of the facility for 7 days. The birds were fed a corn-soybean meal starter diet providing all nutrients at or above the National Research Council (NRC) (1977)

requirements. After 7 days the birds were again sexed with all females being removed.

The chicks were fasted overnight, and weighed to the nearest gram on a Mettler PC2000 balance, wingbanded, and randomly allotted to treatments such that average initial weights were similar for all treatments. Mortality was recorded daily with tissues saved for future analysis. Feed intake data was corrected accordingly when mortalities occurred. The chicks were allotted to 48 separate wire bottomed cages within 2 batteries containing 24 cages each. There were 8 birds allotted to each cage. The chicks were fed and watered ad libitum for the 4 weeks that the animals were on experiment. The birds on experiment 2 averaged 125.4 grams per animal per treatment group. The litter trays beneath each cage were cleaned daily with waterers also cleaned and refilled. Starting environmental temperature for the newly hatched chicks was 35°C with a 3°C drop in temperature per week until an average environmental temperature of 26°C was reached.

II. Experimental Diets

The 48 pigs in experiment 1 were fed corn soybean meal based diets with a single basal diet being mixed at one time, divided into eight equal portions, with treatment concentrations of nickel chloride and iron added at the initiation of the experiment, depending on treatment allocation.

Prior to weaning all pigs were fed a low iron, low nickel creep feed in order to deplete body stores of these elements. The

composition of the creep feed diet is depicted in Table 1. The pigs were fed the creep diet for three weeks at which time they were weaned, weighed, and placed on the corn soybean meal based basal diet, (Table 2.) for 5 days. After 5 days the pigs were allocated to experimental diets. Eight experimental diets were fed in experiment 1. Treatments were replicated 6 times with the amount fed depending upon how much of the ration was consumed at each feeding. The amount of diet fed was increased as each individual pig's consumption increased. The five experimental diets utilized the same basal diet, Table 2, with 4 concentrations of nickel and 2 forms of iron added to the diet, dependent upon treatment, Table 3. Experimental treatments and diet description are given in Table 4. In experiment one, treatment diets 1, 2, 3, and 4 had ferrous iron provided as ferrous sulfate ($FeSo_4 \cdot 7H_2O$). Treatment diets 5, 6, 7, and 8 had ferric iron provided as ferric sulfate ($Fe_2(SO_4)_3 \cdot H2O$). All diets had iron calculated at a concentration of 60 ppm regardless of iron form. Diets 1 and 5 were negative controls and contained 0 ppm nickel. Diets 2 and 6, 3 and 7, and 4 and 8 contained Ni (as nickel chloride) at concentrations of 10 ppm, 50 ppm, and 100 ppm, respectively.

Conformation of diets to calculated nutrient and treatment parameters was confirmed by chemical analysis (Table 5).

The 384 chicks in experiment 2 were fed corn soybean meal based diets with a single basal diet being mixed at one time, divided into sixteen equal portions, and with treatment concentrations of nickel and iron having been added at the initiation of the experiment, depending upon treatment allocation.

TABLE 1. Composition of Pretrial Creep Diet (Experiment 1)

Ingredient	International Reference Number	Percent
No. 2 Yellow Corn	4-02-931	73.26
Soybean Meal (48%)	5-04-604	23.33
Reagent Grade Dicalcium Phosphate		1.27
Reagent Grade Calcium Carbonate		1.01
Salt		0.49
UT-Exp. Premix H-2[a]		0.49
Antibiotic[b]		0.15
Total		100.00

[a]Provided the following per pound pf premix: vitamin A, 300,000 IU; vitamin D, 60,000 Iu; vitamin E, 500 IU; vitamin K compound, 200 mg; riboflavin, 300 mg; nicotinic acid, 1,600 mg; D-pantothenic acid, 1,200 mg; choline, 10,000 mg; vitamin B_{12}, 1.8 mg; zinc, 1.5%; managese, .75% iodine, .055%; copper, .2%; selenium, .002%; antitoxidant, 1.0%.

[b]Plizer Tm 50, 50 g/ton.

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TABLE 2. Composition of Basal Diet (Experiment 1)

Ingredient	International Reference Number	Percent
No. 2 Yellow Corn (9%)	4-02-931	73.28
Soybean Meal (48%)	5-04-604	23.31
Reagent Grade Dicalcium Phosphate		1.26
Reagent Grade Calcium Carbonate		1.02
Salt		0.49
UT-Exp. Premix H-2[a]		0.49
Antibiotic[b]		0.15
Total		100.00

[a]Provided the following per pound of premix: vitamin A, 300,000 IU; vitamin D, 60,000 IU; vitamin E, 500 IU; vitamin K compound, 200 mg; riboflavin, 300 mg; nicotinic acid, 16,00 mg; D-pantothenic acid, 1,200 mg; choline, 10,000 mg; vitamin B_{12}, 1.8 mg; zinc, 1.5%; managese, .75%; iodine, .055%; copper, .2%; selenium, .002%; antioxidant, 1.0%.

[b]Plizer TM 50, 50 g/ton.

TABLE 3. Composition of Experimental Diets (Experiment 1)

Ingredient	International Reference Number	Percent Treatments 1,2,3,4	Percent Treatments 5,6,7,8
No. 2 Yellow Corn (9%)	4-02-931	73.3	73.3
Soybean Meal (48%)	5-04-604	23.3	23.3
Reagent Grade Dicalcium Phosphate		1.3	1.3
Reagent Grade Calcium Carbonate		1.0	1.0
Salt		.5	.5
UT-Exp. Premix H-2[a]		.5	.5
Antibiotic[b]		.1	.1
Nickel Chloride[c]		Variable	Variable
Ferric Sulfate[d]			0.006%
Ferrous Sulfate[e]		0.006%	

[a]Provided the following per pound of premix: vitamin A, 300,000 IU; vitamin D, 60,000 IU; vitamin E, 500 IU; vitamin K compound, 200 mg; riboflavin 300 mg; nicotinic acid, 1,600 mg; D-pantothenic acid, 1,200 mg; choline, 10,000 mg; vitamin B_{12}, 1.8 mg; zinc, 1.5%; manganese, .75%; iodine, .055%; copper, .2%; selenium, .002%; antioxidant, 1.0%.

[b]Pfizer TM 50, 50 g/ton.

[c]Baker, Reagent Grade (purity of 98%).

[d]Kodak, Reagent Grade (purity of 73%).

[e]Fisher, Reagent Grade (purity of 99%).

TABLE 4. Experimental Treatments and Diet Description (Experiment 1)

Treatment	Diet Description
1	Basal + $FeSO_4$[a] + 0 ppm Nickel
2	Basal + $FeSO_4$ + 10 ppm Nickel
3	Basal + $FeSO_4$ + 50 ppm Nickel
4	Basal + $FeSO_4$ + 100 ppm Nickel
5	Basal + $Fe_2(SO_4)_3$[b] + 0 ppm Nickel
6	Basal + $Fe_2(SO_4)_3$ + 10 ppm Nickel
7	Basal + $Fe_2(SO_4)_3$ + 50 ppm Nickel
8	Basal + $Fe_2(SO_4)_3$ + 100 ppm Nickel

[a]$FeSO_4$ calculated to provide iron in the ferrous form at 60 ppm for diets one, two, three, and four.

[b]$Fe_2(SO_4)_3$ calculated to provide iron in the ferric form at 60 ppm for diets five, six, seven, and eight.

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TABLE 5. Analyzed Composition of Experimental Diets (Experiment 1)

Analyzed Components	Fe Form / Fe Level / Ni Level	Experimental Diets 1	2	3	4	5	6	7	8
		Ferrous				Ferric			
		60 (ppm)				60 (ppm)			
		0	10	50	100	0	10	50	100
Dry Matter (%)		88.8	89.2	89.2	89.0	89.3	89.5	89.0	89.3
Ash (%)		4.7	4.7	4.6	5.0	4.6	4.8	4.4	4.6
Crude Protein(%)		21.3	20.8	20.3	19.8	20.4	20.6	20.8	20.2
Crude Fiber (%)		3.0	3.2	3.1	3.0	3.0	3.3	3.4	3.0
Ether Extract (%)		2.1	2.1	1.9	1.8	1.8	2.1	1.7	1.7
Nickel (mg/100g)		.65	1.38	4.81	10.4	.57	1.22	5.75	9.94
Iron (mg/100g)		16.6	17.2	14.4	17.1	14.2	15.6	14.4	14.3
Copper (mg/100g)		1.5	1.5	1.2	1.7	1.2	1.5	1.7	1.1
Zinc (mg/100g)		12.9	15.3	9.2	8.1	12.1	6.8	7.3	8.0
Calcium (%)		.78	.83	.82	.90	.80	.86	.83	.77
Magnesium (%)		.16	.16	.16	.16	.16	.17	.16	.16
Phosphorous (%)		.64	.65	.64	.68	.67	.67	.67	.62

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Prior to the inception of the experiment the chicks were fed a 17% crude protein basal diet (Table 6) for seven days. Sixteen experimental diets were fed in experiment 2. Dietary treatments were replicated 3 times with 8 birds per treatment replicate. The treatments were designed to a 2 X 2 X 4 factorial arrangement utilizing two forms of iron added, two concentrations of iron added, and four concentrations of nickel added depending on the treatment designation. Experimental treatments and diet descriptions are given in Table 7. In experiment 2, treatment diets 1-8 had ferrous iron provided as ferrous sulfate ($FeSo_4$). Treatment diets 9-16 had iron provided as ferric sulfate $Fe_2(SO_4)3$. Iron was provided in either of the two forms at 60 ppm, for diets 1,2,3,4,9,10,11, and 12 or at 100 ppm for diets 5,6,7,8,13,14,15, and 16. Nickel was added to the treatments as nickel chloride ($NiCl_2$) for all diets except diets 1, 5, 9, and 13 which were negative controls and contained no added nickel.

Conformation of the treatment diets in experiment 2 to calculated nutrient and treatment parameters was confirmed by chemical analysis (Tables 8 and 9).

III. Sample Collection

Pigs in experiment 1 were weighed at the beginning of the experiment and thereafter on a weekly basis for four weeks. The animals were bled after overnight fasting via anterior vena cava puncture at the initiation of the experiment and thereafter on days 7, 14, 21, and 28 of the experiment. Feed pans were scrapped

TABLE 6. Composition of Pretest Basal Diet (Experiment 2)

Ingredient	International Reference Number	Percent
Yellow Corn	4-02-935	72.8
Soybean Meal	5-04-612	13.9
Menhaden Fish Meal	5-02-009	5.0
Alfalfa Meal (17% CP)	1-00-023	5.62
Rock Phosphate	6-01-780	1.2
Limestone	6-02-632	.5
Vitamin Mix 6[a]		.6
Salt		.25
Coccidiostat		.10
Manganous Sulfate		.03

[a]Contained per kilogram: vitamin D_3, 125,000 ICU; vitamin A, 700,000 IU; riboflavin, 790 mg; vitamin B_{12}, 1.83 mg; niacin, 6.94 g; D-Ca-panthothenate, 1.22 g; choline, 78 g.

TABLE 7. Experimental Treatments and Diet Description (Experiment 2)

Treatment	Diet Description
1	Basal + 06 ppm $FeSO_4$[a] + 0 ppm Ni as $NiCl_2$[b]
2	Basal + 60 ppm $FeSO_4$ + 10 ppm Ni as $NiCl_2$
3	Basal + 60 ppm $FeSO_4$ + 50 ppm Ni as $NiCl_2$
4	Basal + 60 ppm $FeSO_4$ + 100 ppm Ni as $NiCl_2$
5	Basal + 60 ppm $FeSO_4$ + 0 ppm Ni as $NiCl_2$
6	Basal + 60 ppm $FeSO_4$ + 10 ppm Ni as $NiCl_2$
7	Basal + 60 ppm $FeSO_4$ + 50 ppm Ni as $NiCl_2$
8	Basal + 06 ppm $FeSO_4$ + 100 ppm Ni as $NiCl_2$
9	Basal + 60 ppm $Fe_2(SO_4)_3$[c] + 0 ppm Ni as $NiCl_2$
10	Basal + 60 ppm $Fe_2(SO_4)_3$ + 10 ppm Ni as $NiCl_2$
11	Basal + 60 ppm $Fe_2(SO_4)_3$ + 50 ppm Ni as $NiCl_2$
12	Basal + 60 ppm $Fe_2(SO_4)_3$ + 100 ppm Ni as $NiCl_2$
13	Basal + 60 ppm $Fe_2(SO_4)_3$ + 0 ppm Ni as $NiCl_2$
14	Basal + 60 ppm $Fe_2(SO_4)_3$ + 10 ppm Ni as $NiCl_2$
15	Basal + 60 ppm $Fe_2(SO_4)_3$ + 50 ppm Ni as $NiCl_2$
16	Basal + 60 ppm $Fe_2(SO_4)_3$ + 100 ppm Ni as $NiCl_2$

[a] $FeSO_4$ calculated to provide iron in the ferrous form at 60 ppm for diets 1,2,3,4, and at 100 ppm for diets 5,6,7, and 8.

[b] $NiCl_2$ calculated to provide nickel in the nickelous form for all diets except diet 1, 5, 9, amd 13 which are negative controls and do not contain nickel.

[c] $Fe_2(SO_4)_3$ calculated to provide iron in the ferric form for diets 9 through 16.

TABLE 8. Analyzed Composition of Experimental Diets (Experiment 2)

Analyzed Components	Experimental Diets							
	1	2	3	4	5	6	7	8
Fe Form	Ferrous							
Fe Level	60 (ppm)				100 (ppm)			
Ni Level	0	10	50	100	0	10	50	100
Dry Matter (%)	90.8	90.2	90.1	89.9	90.4	89.9	90.1	89.9
Ash (%)	6.0	6.2	6.2	6.0	5.8	5.5	5.9	5.5
Crude Protein (%)	26.5	26.6	26.9	26.3	25.9	25.2	24.7	26.0
Crude Fiber (%)	3.8	3.9	3.7	3.7	3.6	4.0	3.9	3.9
Ether Extract (%)	8.7	8.3	8.4	8.2	9.1	8.1	8.7	8.8
Nickel (mg/100g)	.70	1.40	6.11	10.2	.72	1.74	5.71	12.2
Iron (mg/100g)	46.1	36.2	41.1	38.2	42.7	41.2	44.0	43.3
Copper (mg/100g)	2.2	1.9	1.9	2.1	1.8	1.9	1.7	1.8
Zinc (mg/100g)	4.3	3.7	3.6	3.6	3.9	3.6	3.6	3.5
Calcium (%)	.87	.80	.98	.87	.86	.78	.85	.83
Magnesium (%)	.20	.19	.19	.19	.19	.19	.19	.19
Phosphorous(%)	.88	.83	.85	.85	.84	.80	.83	.79

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TABLE 9. Analyzed Composition of Experimental Diets (Experiment 2)

Analyzed Components	Experimental Diets							
	9	10	11	12	13	14	15	16
Fe Form	Ferric							
Fe Level	60 (ppm)				100 (ppm)			
Ni Level	0	10	50	100	0	10	50	100
Dry Matter (%)	90.2	90.2	90.4	90.2	90.4	90.4	90.7	90.7
Ash (%)	5.7	6.0	6.2	5.7	5.8	5.8	6.2	6.0
Crude Protein (%)	26.1	25.8	25.7	26.2	26.2	26.7	25.8	25.6
Crude Fiber (%)	3.9	3.8	3.7	3.6	3.5	3.8	3.8	3.9
Ether Extract (%)	8.4	8.4	8.1	8.9	8.6	8.8	9.3	8.8
Nickel (mg/100g)	.66	1.71	6.14	11.5	.64	1.69	5.99	12.20
Iron (mg/100g)	38.9	36.9	35.1	36.3	37.7	38.6	39.4	39.3
Copper (mg/100g)	.9	.9	1.0	1.0	1.0	1.0	1.0	1.0
Zinc (mg/100g)	3.6	3.6	3.6	3.5	3.4	3.5	3.4	3.5
Calcium (%)	.85	.86	.80	.80	.83	.83	.80	.79
Magnesium (%)	.19	.18	.19	.19	.18	.18	.19	.19
Phosphorous (%)	.84	.80	.82	.83	.80	.81	.80	.80

following each feeding and refused feed was dried and weighed in order to calculate a more accurate feed consumption. Representative samples of each of the experimental diets were taken for analysis. Hair samples were taken from each pig on days 14, 21, and 28 of the experiment.

Chicks in experiment 2 were weighed at the start of the experiment and thereafter at weekly intervals for four weeks. Feed consumption was determined on a weekly basis for each treatment replicate. At the termination of experiment 2 all chicks were bled via heart puncture for later analysis. The birds were dissected with 12 tissues, heart, kidney, liver, spleen, pancreas, gonads, brain, skin, breast muscle, leg muscle, feathers, and bone, taken for further analysis. Aliquots of each of the experimental diets were obtained for analysis.

IV. Procedures and Analysis of Samples

The whole blood samples collected from the pigs in experiment 1 were heparinized with sodium heparin, to prevent coagulation, and subjected to further analysis. Blood hemogloblin was determined by the method of Crosby et al., (1954) and hematocrits were determined by the microhematocrit method of McGovern et al., (1955). Red blood cell and white blood cell concentrations were determined by the method of Helleman (1972) on heparinized whole blood after each bleeding. After hemoglobin, hematocrit determination, red blood cell concentration and white cell concentration were ascertained, whole blood samples were centrifuged at an RCF of 3090 g's for ten minutes.

The plasma was removed after centrifugation and prepared for mineral analysis. The plasma samples were storied at -20°C until analyzed for nickel (Ni), iron (Fe), copper (Cu), and zinc (Zn) concentrations. Plasma preparation for mineral analysis consisted of splitting the plasma samples into two equal portions and either diluting 2 ml of plasma with 0.5 ml of a 20% solution of 5'-sulfosalicylic acid (SSA) to precipitate all proteins, or by adding 2 ml of a 20% solution of trichloroacetic acid (TCA) to 1 ml of blood plasma in order to deprotinize the plasma. The plasma from both preparations was then centrifuged at 5000 rpm for 10 minutes and the protein free plasma was collected and analyzed for Ni, Fe, Cu, and Zn concentrations on an Instrumentation Laboratory aa/ae spectrophotometer. The samples were first diluted with glass distilled deionized water until a reading could be obtained using the method described by Instrumentation Laboratory (1978).

Hair samples were taken from each pig on days 14, 21, and 28 of the experiment. Samples were then cleaned by washing in a 1:1000 solution of distilled H_2O and a nonionic detergent (Trition X). The samples were then rinsed with 300 ml of glass distilled water and filtered with a vacuum filter flask and Whatman number 42 ashless filters in a glass funnel. The hair samples were then dried and wet ashed by weighing out a one gram sample and placing it in a 250 ml Phillips beaker and adding 60 ml of reagent grade nitric acid. The solution was heated on a hot plate until dryness was achieved. The flask was allowed to cool and 7 ml of reagent grade perchloric acid (70%) was added and evaporated until approximately 1 ml remained.

This material was then diluted 1:25 with glass distilled water and analyzed spectrophotometrically for nickel, iron, copper, and zinc concentration.

In experiment 2 blood samples were taken and analyzed for hematocrit, hemoglobin, RBC, and plasma concentrations of nickel, iron, copper, and zinc on SSA and TCA treated plasma using the methods described under experiment 1. Tissue samples of heart and kidney were homogenized with three times their weight in water and then wet ashed using the procedure described under experiment 1 for hair samples. The digested tissue samples were then analyzed spectrophotometrically for nickel, iron, copper, and zinc concentrations.

Tissue analysis was performed on an individual animal basis with samples analyzed in a random fashion without regard to treatment or replication.

V. Statistical Analysis of Data

The data were analyzed by the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) (Barr et al., 1979). The GLM procedure is a multivariate regression analysis, determining differences by significant F tests. The Student-Newman-Keuls procedure (SNK) was used to separate means when significant F values indicated differences among means (Steel and Torrie, 1960).

In experiment 1 the independent variables used in the multivariate regression analysis were form of iron, dietary concentration of nickel, and nickel concentration X iron form

interaction. The dependent variables in experiment 1 were weight gain, feed consumption, red blood cell concentration, white blood cell concentration hemoglobin, hematocrit, and blood plasma concentrations of Ni, Fe, Cu, and Zn. Hair concentrations of nickel, iron, copper, and zinc were also dependent varibles that were considered.

In experiment 2 the independent varibles were iron form, iron concentration, nickel concentration and all the 2 and 3 way interactions were also considered in the analysis. The dependent variables for experiment 2 were weight gain, feed consumption, hematocrit, hemoglobin, red cell count, plasma concentrations of nickel, iron, copper, and zinc concentrations and tissue concentrations of Ni, Fe, Cu, and Zn.

CHAPTER IV

RESULTS

I. Experiment 1

Data concerning gain and feed intake of pigs in experiment 1 are presented in Table 10. Gain was not significantly affected by nickel concentration or form of iron at day 7. At days 14, 21, and 28, pigs consuming nickel added at 50 ppm gained significantly less ($P<.05$) than pigs on diets with nickel added at 0, 10, and 100 ppm. Iron form did not have a significant affect on gain over the entire experiment, but there was a trend for decreased gain with ferric iron as compared to experimental treatments utilizing ferrous iron. Feed intake was not significantly affected ($P<.05$) by added nickel concentrations or iron form. There appeared to be a trend for feed intake to decrease as added nickel concentration increased.

Average daily gain (ADG) and feed:gain data are presented in Table 11. Average daily gain was not significantly affected by iron form across all concentrations of nickel. Average daily gain was not significantly affected by added nickel concentration at day 7. However, nickel added at 50 ppm significantly decreased ($P<.05$) average daily gain at days 14, 21, and 28, compared to pigs on nickel added at 0, 10, and 100 ppm. The effect of added nickel was cubic in nature with gain decreasing as nickel concentration increased until at 50 ppm there was a significant decrease in gain. At 100 ppm added nickel gain increased so that it was not significantly different from added concentrations of 0 and 10 ppm nickel. Average daily gain was

TABLE 10. Weekly Gain and Feed Intake Data (Experiment 1)

Iron Form	Day	Nickel Concentration (ppm) 0	10	50	100	Mean	CV
		Gain (Kg)					
Ferrous	7	2.32	2.11	2.00	2.00	2.11	26.9
Ferrous	14	2.79	2.47	2.54	2.42	2.56	19.2
Ferrous	21	2.32	2.20	1.99	2.06	2.15	19.5
Ferrous	28	2.53	2.85	2.53	2.71	2.66	20.7
Ferric	7	2.15[b]	2.20[b]	1.34[a]	1.82[b]	1.91	26.9
Ferric	14	2.79[b]	2.47[b]	1.69[a]	2.45[b]	2.38	19.2
Ferric	21	2.21[b]	2.35[b]	0.94[a]	2.26[b]	1.97	19.5
Ferric	28	2.71[b]	2.68[b]	1.53[a]	2.77[b]	2.45	20.7
Mean							
	7	2.24	2.16	1.70	1.92		
	14	2.79[b]	2.47[b]	2.15[a]	2.43[b]		
	21	2.26[b]	2.28[b]	1.51[a]	2.15[b]		
	28	2.62[b]	2.76[b]	2.09[a]	2.74[b]		

Iron Form	Day	0	10	50	100	Mean	CV
		Feed (Kg)					
Ferrous	7	3.84	3.68	3.56	3.38	3.62	15.3
Ferrous	14	5.07	4.98	4.80	4.58	4.86	12.3
Ferrous	21	5.38	4.92	5.07	4.45	4.96[c]	22.9
Ferrous	28	6.41	6.40	6.52	6.00	6.33	12.0
Ferric	7	3.79	3.75	3.14	3.55	3.57	15.3
Ferric	14	5.01	5.11	4.50	4.72	4.85	12.3
Ferric	21	4.76	4.35	3.65	3.80	4.18[d]	22.9
Ferric	28	6.30	6.60	5.37	6.33	6.18	12.0
Mean							
	7	3.81	3.71	3.38	3.46		
	14	5.04	5.04	4.66	4.64		
	21	5.08	4.63	4.43	4.15		
	28	6.35	6.50	6.00	6.15		

[a,b]Means in rows with different superscripts differ ($P<.05$).
[c,d]Means in corresponding rows with different superscripts differ ($P<.05$).

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TABLE 11. Average Daily Gain and Feed: Gain Data (Experiment 1)

Iron Form	Day	Nickel Concentration (ppm) 0	10	50	100	Mean	CV
		ADG (Kg)					
Ferrous	7	.33	.30	.29	.29	.30	26.9
Ferrous	14	.40	.35	.36	.35	.37	19.2
Ferrous	21	.33	.31	.28	.29	.31	19.5
Ferrous	28	.36	.41	.37	.39	.38	20.7
Ferric	7	.31	.31	.20	.26	.27	26.9
Ferric	14	.40[b]	.35[b]	.24[a]	.35[b]	.34	19.2
Ferric	21	.32[b]	.34[b]	.13[a]	.32[b]	.28	19.5
Ferric	28	.39[b]	.38[b]	.22[a]	.40[b]	.35	20.7
Mean							
	7	.32	.31	.24	.27		
	14	.40[b]	.35[b]	.31[a]	.35[b]		
	21	.32[b]	.33[b]	.22[a]	.31[b]		
	28	.37[b]	.39[b]	.30[a]	.39[b]		

Iron Form	Day	0	10	50	100	Mean	CV
		F/G					
Ferrous	7	.85	.80	.82	.77	.81	57.0
Ferrous	14	.83	.95	.87	.88	.88[c]	14.4
Ferrous	21	1.05	1.05	1.18	1.01	1.07	32.3
Ferrous	28	1.17	1.04	1.24	1.02	1.12	20.9
Ferric	7	.83[b]	.78[b]	1.07[b]	1.51[a]	1.02	57.0
Ferric	14	.81[b]	.94[b]	1.24[a]	.89[b]	.96[d]	14.4
Ferric	21	.98[b]	.89[b]	1.70[a]	.79[b]	1.07	32.3
Ferric	28	1.07[b]	1.18[b]	1.60[a]	1.08[b]	1.22	20.9
Mean							
	7	.84	.79	.93	1.11		
	14	.82[b]	.95[b]	1.04[a]	.89[b]		
	21	1.02[b]	.97[b]	1.42[a]	.91[b]		
	28	1.12[b]	1.11[b]	1.41[a]	1.04[b]		

[a,b]Means in rows with different superscripts differ (P<.05)
[c,d]Means in corresponding rows with different superscripts differ (P<.05).

significantly decreased (P<.05) with ferric iron at added nickel concentration of 50 ppm but not ferrous iron at nickel concentration of 50 ppm. ADG was not significantly affected by form of iron across all concentrations of nickel. The interaction between nickel concentration and iron was significant (P<.05) for average daily gain at days 21, and 28 but not at days 7, and 14. Pigs receiving ferric iron gained less at 50 ppm nickel while pigs receiving ferrous iron at 50 ppm Ni gained similar to pigs fed other concentrations of nickel.

Data for red blood cell concentrations (RBC) and for white cell concentrations are presented in Table 12. Red blood cell concentration was not significantly affected by form of iron across all nickel concentrations. Nickel added at 50 ppm significantly (P<.05) increased RBC at day 28, but not at days 7, 14, or 21. At day 28 pig fed ferric iron had a significantly higher RBC concentration for nickel added at 50 ppm than pigs fed ferrous iron. White blood cell concentration was not significantly affected by added dietary concentrations of Ni or Fe or by form of iron.

Hematocrit (Hct) and hemoglobin (Hb) data are presented in Table 13. Hematocrit was significantly (P<.05) increased by nickel added at 50 ppm at day 28 but not at days 7, 14, and 21. The interaction between nickel concentration (at 50 ppm) and ferric iron was significant for hematocrit at day 28 but not days 7, 14, or 21. Hemoglobin was not significantly affected by nickel concentration or form of iron. There was a tendency for hemoglobin to be increased at

TABLE 12. Red Cell Count and White Cell Count (Experiment 1)

Iron Form	Day	Nickel Concentration (ppm) 0	10	50	100	Mean	SEM
		RBC (10^6/mm^3)					
Ferrous	7	8.17	5.97	7.32	6.62	7.02	.64
Ferrous	14	6.05	6.46	6.65	6.38	6.38	.41
Ferrous	21	6.05	6.33	6.12	6.35	6.21	.19
Ferrous	28	6.83	7.03	6.82	6.70	6.85	.23
Ferric	7	6.00	6.80	6.68	6.82	6.58	.89
Ferric	14	6.98	6.28	7.05	5.93	6.56	.52
Ferric	21	5.92	6.33	6.82	6.07	6.28	.39
Ferric	28	6.93[b]	6.75[b]	8.48[a]	6.83[b]	7.25	.46
Mean							
	7	7.08	6.38	7.00	6.72		
	14	6.52	6.36	6.85	6.16		
	21	5.98	6.33	6.47	6.21		
	28	6.88[b]	6.89[b]	7.65[a]	6.77[b]		

Iron Form	Day	0	10	50	100	Mean	SEM
		WBC (10^3/mm^3)					
Ferrous	7	16.57	22.17	20.05	19.40	19.55	2.03
Ferrous	14	18.35	20.88	19.64	19.80	19.74	1.60
Ferrous	21	16.30	21.18	16.73	17.68	17.98	1.42
Ferrous	28	16.77	20.35	14.95	17.00	17.27	1.77
Ferric	7	19.02	17.95	19.73	17.80	18.63	1.99
Ferric	14	19.24	19.74	18.50	17.30	18.77	2.43
Ferric	21	16.13	17.25	15.47	19.55	17.10	2.57
Ferric	28	16.05	17.58	18.05	16.82	17.13	1.29
Mean							
	7	17.79	20.06	19.89	18.60		
	14	18.84	20.31	19.07	18.69		
	21	16.22	19.22	16.10	18.62		
	28	16.41	18.97	16.50	16.91		

[a,b]Means in rows with different superscripts differ (P<.05).

TABLE 13. Hematocrit and Hemoglobin Concentration (Experiment 1)

Iron Form	Day	Nickel Concentration (ppm) 0	10	50	100	Mean	SEM
		Hct (%RBC)					
Ferrous	Initial	38.9	39.0	39.8	38.8	39.1	1.08
Ferrous	7	39.1	38.7	39.2	37.9	38.7	1.25
Ferrous	14	38.8	36.5	36.5	40.1	38.0	2.39
Ferrous	21	45.4	54.0	44.3	42.7	46.6	3.58
Ferrous	28	38.0	37.5	37.6	38.4	37.9	.73
Ferric	Initial	39.8	41.9	38.8	38.8	39.8	1.42
Ferric	7	39.0	38.3	42.1	39.7	39.8	.85
Ferric	14	38.5	36.2	44.0	39.9	39.6	1.58
Ferric	21	50.7	48.1	48.3	43.0	47.5	3.74
Ferric	28	37.7^{b}	37.2^{b}	44.0^{a}	36.5^{b}	38.8	1.43
Mean							
	Initial	39.3	40.5	39.3	38.8		
	7	39.1	38.5	40.6	38.8		
	14	38.6	36.3	40.2	40.0		
	21	48.1	51.0	46.3	42.9		
	28	37.8^{b}	37.3^{b}	40.8^{a}	37.5^{b}		

Iron Form	Day	0	10	50	100	Mean	SEM
		Hb (g/100ml)					
Ferrous	Initial	14.05	13.67	13.97	13.83	13.88	.67
Ferrous	7	13.80	13.50	14.02	15.82	14.28	.57
Ferrous	14	13.93	13.83	15.87	14.35	14.50	1.09
Ferrous	21	14.57	14.95	14.67	16.38	15.14	1.44
Ferrous	28	13.93	13.45	13.60	14.43	13.85	.45
Ferric	Initial	15.37	14.80	14.25	13.12	14.38	.51
Ferric	7	14.10	13.47	15.03	14.15	14.19	.44
Ferric	14	12.67	14.18	15.75	13.97	14.14	.52
Ferric	21	17.70	15.95	17.32	16.82	16.95	1.67
Ferric	28	13.77	12.88	15.37	12.85	13.72	.56
Mean							
	Initial	14.71	14.23	14.11	13.48		
	7	13.95	13.48	14.53	14.98		
	14	13.30	14.01	15.81	14.16		
	21	16.13	15.45	15.99	16.60		
	28	13.85	13.17	14.48	13.64		

^{a,b}Means in rows with different superscripts differ ($P<.05$).

higher concentrations of nickel when pigs received ferric iron but not to as great an extent with ferrous iron.

Nickel and iron concentrations of hair samples taken from pigs on experiment 1 are presented in Table 14. Nickel and iron contents of hair were not significantly affected by nickel concentration or iron form at days 7, 14, 21, or 28. Copper (Cu) and zinc (Zn) concentration in hair samples are presented in Table 15. Added nickel concentrations and form of iron did not have a significant affect on copper and zinc concentration in hair samples.

Results of nickel and iron concentrations of sulfosalicylic acid treated plasma are presented in Table 16. Nickel concentration of sulfosalicylic acid treated plasma was not significantly affected by form of iron across all dietary concentrations of nickel. Added nickel at 100 ppm significantly increased ($P<.05$) nickel concentration of SSA treated plasma at day 21 but not at days 7, 14, and 28.

Nickel concentration of SSA treated plasma was significantly increased ($P<.05$) by ferric iron at days 21, and 28 for nickel added at 100 ppm but not for days 7 and 14. Iron concentrations of SSA treated plasma were significantly increased ($P<.05$) by ferric iron across all added concentrations of nickel at days 21, and 28 but not days 7 and 14. Nickel concentration added at 50 ppm significantly increased ($P<.05$) iron concentration for SSA treated plasma over nickel concentrations of 0, 10, and 100 ppm for days 21, and 28 but not for days 7 and 14. Iron concentration for nickel concentration at 100 ppm was significantly higher ($P<.05$) than added nickel at 10

TABLE 14. Hair Concentrations of Nickel and Iron (Experiment 1)

Iron Form	Day	Nickel Concentration (ppm) 0	10	50	100	Mean	SEM
		Nickel (mg/100g)					
Ferrous	14	.263	.206	.176	.207	.213	.013
Ferrous	21	.233	.229	.246	.298	.252	.027
Ferrous	28	.196	.210	.197	.203	.201	.011
Ferric	14	.167	.150	.189	.159	.166	.024
Ferric	21	.193	.237	.244	.235	.227	.003
Ferric	28	.180	.183	.185	.231	.195	.007
Mean							
	14	.215	.178	.183	.181		
	21	.213	.233	.245	.266		
	28	.188	.197	.191	.217		

Iron Form	Day	0	10	50	100	Mean	SEM
		Iron (mg/100g)					
Ferrous	14	4.293	3.917	3.441	4.623	4.044	.091
Ferrous	21	3.977	3.612	3.678	4.383	3.912	.423
Ferrous	28	3.372	3.741	3.077	2.858	3.260	.067
Ferric	14	3.986	3.415	6.167	3.246	4.203	.004
Ferric	21	3.530	3.280	4.916	3.829	3.889	.221
Ferric	28	3.262	2.554	3.577	3.469	3.214	.014
Mean							
	14	4.139	3.666	4.804	3.872		
	21	3.753	3.446	4.297	4.106		
	28	3.317	3,148	3.324	3.164		

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TABLE 15. Hair Concentrations of Copper and Zinc (Experiment 1)

Iron Form	Week	Nickel Concentration (ppm) 0	10	50	100	Mean	SEM
		Copper (mg/100g)					
Ferrous	1	1.715	1.652	1.664	1.569	1.653	.007
Ferrous	2	1.429	1.395	1.417	1.330	1.393	.024
Ferrous	3	1.412	1.431	1.427	1.369	1.410	.003
Ferric	1	1.648	1.687	1.668	1.712	1.679	.001
Ferric	2	1.402	1.476	1.385	1.496	1.440	.001
Ferric	3	1.413	1.464	1.403	1.481	1.440	.056
Mean							
	1	1.681	1.669	1.666	1.647		
	2	1.416	1.435	1.401	1.413		
	3	1.413	1.448	1.415	1.425		

Iron Form	Week	0	10	50	100	Mean	SEM
		Zinc (mg/100g)					
Ferrous	1	19.966	18.985	17.501	20.018	19.078	.624
Ferrous	2	18.957	18.524	18.030	18.724	18.559	1.747
Ferrous	3	18.456	17.559	17.301	17.347	17.666	1.321
Ferric	1	18.378	17.766	19.772	18.304	18.555	.891
Ferric	2	17.938	18.456	19.577	18.851	18.705	1.419
Ferric	3	17.605	17.104	18.154	17.816	17.670	1.273
Mean							
	1	19.171	18.376	18.636	19.083		
	2	18.447	18.490	18.804	18.787		
	3	18.031	17.332	17.727	17.582		

TABLE 16. Nickel and Iron Concentration-Sulfosalicylic Acid Treated Plasma (Experiment 1)

Iron Form	Day	Nickel Concentrations (ppm) 0	10	50	100	Mean	SEM
		Nickel (ppm)					
Ferrous	Initial	.15	.14	.17	.15	.15	.002
Ferrous	7	.12	.15	.16	.16	.15	.001
Ferrous	14	.15	.15	.16	.16	.15	.001
Ferrous	21	.14	.16	.16	.17	.16	.006
Ferrous	28	.18	.18	.19	.17	.18	.001
Ferric	Initial	.14	.14	.17	.14	.15	.017
Ferric	7	.16	.15	.15	.18	.16	.003
Ferric	14	.16	.13	.16	.18	.16	.001
Ferric	21	.13[b]	.14[b]	.15[b]	.17[a]	.15	.005
Ferric	28	.18[b]	.21[b]	.17[b]	.25[a]	.20	.003
Mean							
	Initial	.14	.14	.17	.14		
	7	.14	.15	.16	.17		
	14	.15	.14	.16	.17		
	21	.14[b]	.15[b]	.16[b]	.17[a]		
	28	.18	.19	.18	.21		

Iron Form	Day	0	10	50	100	Mean	SEM
		Iron (ppm)					
Ferrous	Initial	1.45	1.25	1.70	1.67	1.50	.002
Ferrous	7	1.76	1.67	1.91	1.78	1.78	.014
Ferrous	14	1.49	1.48	1.88	1.60	1.61	.002
Ferrous	21	1.18[c]	1.23[c]	1.34[b]	1.54[a]	1.32[d]	.001
Ferrous	28	1.34[b]	1.28[b]	1.50[b]	1.64[a]	1.44[d]	.011
Ferric	Initial	1.31	1.49	1.76	1.48	1.51	.003
Ferric	7	1.76	1.53	2.06	1.97	1.82	.002
Ferric	14	1.40	1.50	2.08	1.71	1.65	.131
Ferric	21	1.44[b,c]	1.24[c]	2.29[a]	1.55[b]	1.63[e]	.072
Ferric	28	1.45[c]	1.56[c]	2.64[a]	1.92[b]	1.89[e]	.066
Mean							
	Initial	1.37	1.37	1.73	1.57		
	7	1.76	1.59	1.97	1.87		
	14	1.45	1.49	1.97	1.66		
	21	1.31[b,c]	1.24[c]	1.82[a]	1.54[b]		
	28	1.40[c]	1.42[c]	2.07[a]	1.78[b]		

[a,b,c]Means within rows with different superscripts differ ($P<.05$).
[d,e]Means in corresponding rows with different superscripts differ ($P<.05$).

ppm for days 21 and 28 but not at 0 added concentrations for day 21, but was significantly higher ($P<.05$) than for 0 added Ni at day 28.

Data concerning copper and zinc concentrations of SSA treated plasma are presented in Table 17. Ferric iron significantly increased ($P<.05$) Cu concentration at days 21 and 28, but not for days 7 and 14, across all concentrations of added nickel. Added nickel concentration at 50 ppm resulted in a significant increase ($P<.05$) in copper concentrations for days 14, 21, and 28, but not for day 7 or the initial bleeding. Ferric iron increased Cu concentration for weeks 14, 21, and 28 for nickel added at 50 ppm but not for day 7 or the initial bleeding. Zinc concentrations of SSA treated plasma were not significantly affected by form of iron or added nickel concentrations. Added concentration of nickel tended to decrease plasma zinc concentrations by a small amount but not significantly.

Nickel and iron concentrations of trichloroacetic acid (TCA) treated plasma are presented in Table 18. Nickel concentration was not significantly affected by form of iron or nickel concentration. Ferric iron significantly ($P<.05$) increased iron concentration of TCA treated plasma for days 21 and 28 but not days 7 and 14 across all concentrations of nickel. Added nickel at 50 ppm significantly increased ($P<.05$) iron concentration at days 14, 21 and 28 but not for day 7 or the initial bleeding. Ferric iron significantly increased iron concentration at days 14, 21 and 28, for nickel added at 50 ppm compared to ferrous iron for nickel added at 50 ppm.

TABLE 17. Plasma Copper and Zinc Concentration (SSA) (Experiment 1)

		Plasma Copper Concentration (ppm)					
		Nickel Concentration (ppm)					
Iron Form	Day	0	10	50	100	Mean	SEM
Ferrous	Initial	1.69	1.70	1.69	1.71	1.69	.017
Ferrous	7	1.63	1.60	1.71	1.72	1.67	.003
Ferrous	14	1.77	1.20	1.23	1.15	1.19	.044
Ferrous	21	1.38	1.37	1.35	1.39	1.37[c]	.005
Ferrous	28	1.58	1.56	1.51	1.51	1.54[c]	.066
Ferric	Initial	1.62	1.71	1.73	1.52	1.65	.001
Ferric	7	1.52	1.73	1.74	1.83	1.70	.001
Ferric	14	1.15[b]	1.27[b]	1.43[a]	1.24[b]	1.27	.022
Ferric	21	1.36[b]	1.44[b]	1.76[a]	1.45[b]	1.50[d]	.013
Ferric	28	1.47[b]	1.45[b]	2.06[a]	1.69[b]	1.69[d]	.177
Mean							
	Initial	1.65	1.70	1.71	1.60		
	7	1.57	1.67	1.72	1.77		
	14	1.16[b]	1.23[b]	1.32[a]	1.19[b]		
	21	1.37[b]	1.40[b]	1.55[a]	1.42[b]		
	28	1.53[b]	1.51[b]	1.78[a]	1.61[b]		
		Plasma Zinc Concentration (ppm)					
Iron Form	Day	0	10	50	100	Mean	SEM
Ferrous	Initial	.83	.82	.79	.80	.81	.001
Ferrous	7	1.20	1.02	1.05	1.02	1.06	.024
Ferrous	14	1.27	1.25	1.04	1.13	1.16	.024
Ferrous	21	1.35	1.30	1.20	1.28	1.28	.005
Ferrous	28	1.35	1.29	1.18	1.21	1.26	.036
Ferric	Initial	.91	.80	.73	.75	.80	.001
Ferric	7	1.11	1.11	1.08	.98	1.07	.022
Ferric	14	1.19	1.07	1.27	1.31	1.19	.016
Ferric	21	1.26	1.27	1.20	1.22	1.24	.078
Ferric	28	1.36	1.30	1.32	1.22	1.30	.030
Mean							
	Initial	.88	.81	.76	.77		
	7	1.14	1.07	1.07	1.00		
	14	1.22	1.15	1.14	1.20		
	21	1.30	1.28	1.20	1.25		
	28	1.36	1.29	1.23	1.22		

[a,b]Means in rows with different superscripts differ ($P<.05$).
[c,d]Means in corresponding rows with different superscripts differ ($P<.05$).

TABLE 18. Plasma Nickel and Iron Concentration (TCA) (Experiment 1)

		Plasma Nickel Concentration (ppm)					
		Nickel Level (ppm)					
Iron Form	Day	0	10	50	100	Mean	SEM
Ferrous	Initial	.27	.25	.28	.26	.26	.04
Ferrous	7	.37	.32	.39	.38	.36	.01
Ferrous	14	.33	.33	.37	.35	.34	.01
Ferrous	21	.34	.36	.32	.33	.34	.01
Ferrous	28	.30	.36	.34	.36	.34	.03
Ferric	Initial	.26	.24	.25	.30	.26	.02
Ferric	7	.35	.35	.34	.40	.36	.02
Ferric	14	.34	.32	.33	.35	.34	.01
Ferric	21	.33	.33	.35	.34	.34	.03
Ferric	28	.36	.34	.36	.36	.36	.06
Mean							
	Initial	.27	.24	.26	.28		
	7	.36	.34	.36	.39		
	14	.33	.33	.35	.35		
	21	.33	.35	.33	.33		
	28	.33	.35	.34	.36		

Iron Form	Day	Plasma Iron Concentration					
Ferrous	Initial	2.04	1.71	2.20	2.08	1.99	.07
Ferrous	7	2.11	2.04	2.34	2.18	2.16	.05
Ferrous	14	2.08	2.11	2.47	2.17	2.20	.14
Ferrous	21	1.63	1.63	1.76	1.97	1.74^{c}	.28
Ferrous	28	1.18	1.75	2.09	2.06	1.92^{c}	.06
Ferric	Initial	1.86	2.05	2.42	2.08	2.10	.11
Ferric	7	2.10	1.80	2.41	2.25	2.15	.03
Ferric	14	1.93^{b}	1.97^{b}	2.89^{a}	2.24^{b}	2.27	.01
Ferric	21	1.88^{b}	1.78^{b}	2.79^{a}	2.03^{b}	2.14^{d}	.02
Ferric	28	1.96^{b}	1.98^{b}	2.87^{a}	2.34^{b}	2.30^{d}	.01
Mean							
	Initial	1.95	1.85	2.31	2.08		
	7	2.10	1.94	2.37	2.21		
	14	2.00^{b}	2.05^{b}	2.68^{a}	2.20^{b}		
	21	1.75^{b}	1.69^{b}	2.28^{a}	2.00^{b}		
	28	1.89^{b}	1.84^{b}	2.48^{a}	2.20^{b}		

^{a,b}Means in rows with different superscripts differ (P<.05).

^{c,d}Means in corresponding rows with different superscripts differ (P<.05).

Copper and zinc concentrations of TCA treated plasma are represented in Table 19. Copper concentration was significantly higher ($P<.05$) for pigs treated with ferric iron compared to pigs treated with ferrous iron at days 21 and 28, but not days 7 and 14 across all concentrations of nickel. Copper concentration was significantly higher ($P<.05$) for TCA treated plasma with nickel added at 50 ppm for days 14, 21, and 28, but not for day 7. Ferric iron significantly increased ($P<.05$) copper concentration at days 14, 21, and 28 for nickel added at 50 ppm compared to ferrous iron for nickel added during the same periods. Zinc concentration was not affected by form of iron or added nickel concentrations.

II. Experiment 2

The effect of iron form and iron concentration on performance data are presented in Table 20. Average daily gain (ADG) was higher ($P<.05$) with iron supplemented chick diets at 60 ppm than iron added at 100 ppm for days 7 and 14. Average daily gain was higher ($P<.05$) at day 21 for iron supplemented at 100 ppm than for iron added at 60 ppm. Average daily gain was not affected by iron concentration on day 28 or for total average daily gain. Iron form did not affect ADG on days 7 and 21. On day 14 ferrous iron supplemented chicks had a significantly higher ($P<.05$) average gain than did those fed the ferric iron supplemented diets. ADG on day 28 was higher ($P<.05$) for chicks fed a ferric iron supplemented diet than for those fed a ferrous iron supplemented diet. Total average daily gain was not affected by form of iron. Chicks fed diets supplemented with iron at

TABLE 19. Plasma Copper and Zinc Concentration (TCA) (Experiment 1)

		Plasma Copper Concentration (ppm)					
		Nickel Concentration (ppm)					
Iron Form	Day	0	10	50	100	Mean	SEM
Ferrous	Initial	1.80	1.72	1.78	1.80	1.77	.021
Ferrous	7	1.69	1.65	1.68	1.71	1.68	.009
Ferrous	14	1.49	1.51	1.48	1.52	1.50	.133
Ferrous	21	1.50	1.52	1.44	1.48	1.48[c]	.047
Ferrous	28	1.46	1.50	1.46	1.48	1.47[c]	.015
Ferric	Initial	1.81	1.96	2.04	1.88	1.92	.022
Ferric	7	1.51	1.65	1.77	1.80	1.68	.153
Ferric	14	1.37[b]	1.53[b]	1.90[a]	1.60[b]	1.60	.027
Ferric	21	1.40[b]	1.52[b]	1.91[a]	1.64[b]	1.61[d]	.164
Ferric	28	1.52[b]	1.48[b]	2.17[a]	1.58[b]	1.67[d]	.283
Mean							
	Initial	1.18	1.84	1.91	1.84		
	7	1.60	1.65	1.73	1.75		
	14	1.43[b]	1.52[b]	1.69[a]	1.56[b]		
	21	1.45[b]	1.52[b]	1.68[a]	1.56[b]		
	28	1.49[b]	1.49[b]	1.78[a]	1.53[b]		
		Plasma Zinc Concentration (ppm)					
Iron Form	Day	0	10	50	100	Mean	SEM
Ferrous	Initial	.88	.98	.94	1.36	1.03	.005
Ferrous	7	1.28	1.12	1.09	1.05	1.13	.007
Ferrous	14	1.25	1.23	1.06	1.12	1.16	.123
Ferrous	21	1.44	1.39	1.32	1.36	1.38	.041
Ferrous	28	1.25	1.30	1.20	1.19	1.23	.002
Ferric	Initial	1.05	1.02	.90	.96	.99	.001
Ferric	7	1.13	1.14	1.11	1.41	1.20	.072
Ferric	14	1.24	1.13	1.52	1.12	1.25	.009
Ferric	21	1.31	1.41	1.25	1.16	1.28	.179
Ferric	28	1.37	1.26	1.16	1.27	1.26	.084
Mean							
	Initial	.96	1.00	.92	1.14		
	7	1.20	1.13	1.10	1.23		
	14	1.24	1.18	1.29	1.12		
	21	1.37	1.40	1.28	1.26		
	28	1.30	1.28	1.18	1.22		

[a,b]Means in rows with different superscripts differ ($P<.05$).
[c,d]Means in corresponding rows with different superscripts differ ($P<.05$).

TABLE 20. Effect of Iron Form and Iron Level on Chick Performance (g/day) (Experiment 2)

Item	Day	Iron Form		P		Iron Level (ppm)		P	
		Ferrous	Ferric	Value	CV	60	100	Value	CV
ADG1	7	22.38	21.97	.226	16.0	22.76[c]	21.59[d]	.001	16.0
ADG2	14	18.66[a]	16.70[b]	.001	26.7	18.72[c]	16.62[d]	.001	26.7
ADG3	21	31.39	30.98	.596	22.9	30.31	32.07[d]	.015	22.9
ADG4	28	28.11[a]	29.95[b]	.031	28.0	28.41	29.66	.135	28.0
TADG		25.14	25.00	.731	15.0	25.05	25.09	.912	15.0
ADF1	7	32.23	32.08	.812	10.0	32.75	31.52	.082	10.0
ADF2	14	44.78[a]	41.75[b]	.009	8.1	45.30[c]	41.22[d]	.001	8.1
ADF3	21	68.43	65.68	.630	9.8	66.39	67.34	.737	9.8
ADF4	28	71.96	72.48	.854	10.4	71.59	72.96	.490	10.4
TADF		53.55	53.56	.343	6.0	53.61	53.69	.056	6.0
FD/gain	7	1.44	1.46	.460	5.3	1.44	1.45	.340	5.3
FD/gain	14	2.40	2.50	.340	14.8	2.42	2.48	.578	14.8
FD/gain	21	2.18	2.12	.585	16.0	2.19	2.10	.363	16.0
FD/gain	28	2.56	2.42	.167	14.0	2.52	2.46	.568	14.0
TFD/gain		2.13	2.14	.880	6.8	2.14	2.14	.999	6.8

[a,b,c,d]Means in rows within major headings with different superscripts differ (P<.05).

60 ppm had a significantly higher (P<.05) average daily feed intake (ADF) at day 7 and day 14. Average daily feed intake was not significantly affected by concentration of iron on day 21, 28, or for total average daily feed intake for the experiment. Diets supplemented with ferrous iron had a significantly higher (P<.05) average daily feed intake than those supplemented with ferric iron, for days 7 and 14 of the experiment. Average daily feed intake was not significantly affected by form of iron for days 21, 28, or for total ADF. Feed:gain was not significantly affected by form of iron or concentration of iron.

The effects of iron form and iron concentration on hematological data are presented in Table 21. Hematocrit was not significantly affected by iron form or iron concentration. Hemoglobin was not affected by concentration of iron. Hemoglobin content was significantly higher (P<.05) for those chicks supplemented with ferric iron compared to ferrous iron supplemented diets. The nickel, iron, and zinc concentrations in sulfosalicylic acid treated blood plasma were not significantly affected by form or concentration of iron. Copper concentration in SSA treated plasma was significantly higher (P<.05) for ferric iron treated diets than for ferrous iron supplemented diets. Nickel concentration for trichloroacetic acid (TCA) treated blood plasma was not significantly affected by concentration of iron. Nickel concentration in TCA treated plasma was significantly increased (P<.05) for chick diets supplemented with ferrous iron compared to those animals on ferric iron supplemented diets. Iron concentration was significantly higher (P<.05) for

TABLE 21. Effect of Iron Form and Iron Level on Hematology and Plasma Minerals at 28 Days. (Experiment 2)

Item	Units	Iron Form		P		Iron Level (ppm)		P	
		Ferrous	Ferric	Value	CV	60	100	Value	CV
Hct	% RBC	28.14	28.36	.378	10.7	28.11	28.41	.280	10.7
Hb	g/100ml	8.63[a]	8.97[b]	.017	15.0	8.74	8.87	.305	15.0
Plasma-SSA	(mg/kg)								
Nickel		.21	.20	.428	13.4	.20	.21	.092	13.4
Iron		.96	.91	.155	12.7	.94	.94	.979	12.7
Copper		.080[a]	.084[b]	.021	11.6	.082	.082	.263	11.6
Zinc		1.63	1.75	.131	15.6	1.67	1.71	.569	15.6
Plasma-TCA	(mg/kg)								
Nickel		.39[a]	.37[b]	.013	20.2	.38	.38	.519	20.2
Iron		1.52[a]	1.40[b]	.003	21.8	1.51[c]	1.40[d]	.002	21.8
Copper		.11	.11	.348	31.7	.12[c]	.10[d]	.001	31.7
Zinc		1.77	1.72	.181	22.2	1.75	1.74	.924	22.2

[a,b,c,d]Means in rows within major headings with different superscripts differ (P<.05).

chicks fed added iron concentration of 60 ppm compared to those chicks fed 100 ppm added iron. Iron concentration was significantly higher ($P<.05$) for chicks supplemented with ferrous iron compared to those supplemented with ferric iron. Iron form did not have a significant effect on copper or zinc concentration. Copper concentration was significantly higher ($P<.05$) for TCA treated plasma at 60 ppm added iron compared to added dietary iron concentrations of 100 ppm iron. Zinc concentration was significantly higher ($P<.05$) for TCA treated plasma at added iron concentrations of 60 ppm compared to added concentrations at 100 ppm.

Effect of iron form and iron concentration on kidney and heart tissue concentrations of nickel, iron, copper and zinc on a wet and dry tissue basis are presented in Tables 22 and 23. Iron form and iron concentration did not have a significant effect on nickel, iron, copper, and zinc concentrations of kidney and heart tissue on a wet or dry basis. There appeared to be a trend for higher concentrations of kidney and heart tissue concentrations of nickel, iron, copper, and zinc for diets supplemented with ferric iron compared to diets supplemented with ferrous iron.

The effects of dietary nickel on performance and hematology are presented in Tables 24 and 25. Average daily gain was significantly increased ($P<.05$) by nickel added at 50 and 100 ppm at day 7, but on day 14 ADG was significantly decreased ($P<.05$) by nickel added at 100 ppm. There appeared to be a trend toward decreased ADG as nickel concentration increased at day 14. Average daily gain was not significantly decreased ($P >.05$) at day 21 by added nickel

TABLE 22. Effect of Iron Form and Iron Level on Tissue Mineral Levels (mg/kg) (Experiment 2)

Kidney	Iron Form		P		Iron Level (ppm)		P	
	Ferrous	Ferric	Value	CV	60	100	Value	CV
Nickel Wet	.08	.11	.153	89.0	.11	.11	.278	89.0
Nickel Dry	.35	.41	.241	54.8	.38	.38	.882	54.8
Iron Wet	4.24	5.76	.139	65.8	5.35	4.70	.502	65.8
Iron Dry	19.59	22.65	.275	47.1	20.24	21.82	.821	47.1
Copper Wet	.34	.31	.832	92.6	.28	.38	.338	92.6
Copper Dry	1.23	1.47	.208	53.2	1.30	1.39	.868	53.2
Zinc Wet	1.38	1.74	.085	47.4	1.47	1.63	.667	47.4
Zinc Dry	6.37	8.15	.080	50.0	6.94	7.50	.809	50.0

TABLE 23. Effect of Iron Form and Iron Level on Tissue Mineral Levels (mg/kg) (Experiment 2)

Heart	Iron Form		P		Iron Level (ppm)		P	
	Ferrous	Ferric	Value	CV	60	100	Value	CV
Nickel Wet	.72	.75	.419	42.1	.75	.72	.454	42.1
Nickel Dry	4.01	3.80	.604	95.5	4.07	3.72	.378	95.5
Iron Wet	21.69	22.18	.540	34.7	21.99	21.89	.969	34.7
Iron Dry	110.43	114.84	.296	38.7	111.49	113.84	.590	38.7
Copper Wet	2.26	2.27	.973	69.5	2.16	2.38	.181	69.5
Copper Dry	11.38	11.26	.774	35.0	11.44	11.19	.774	35.0
Zinc Wet	9.14	9.12	.752	24.0	9.26	8.99	.273	24.0
Zinc Dry	46.67	47.81	.444	27.9	47.53	46.90	.678	27.9

TABLE 24. Effect of Nickel Level on Performance (Experiment 2)

Item	Day	Nickel Level (ppm) 0	10	50	100	P Value	CV
ADG-g/day							
ADG1	7	21.05	21.94[b]	22.86[a]	22.86[a]	.001	16.0
ADG2	14	18.66[b]	17.97[b]	17.18[b]	16.89[a]	.049	26.7
ADG3	21	32.31	30.10	31.29	31.07	.200	22.9
ADG4	28	28.86	29.43	28.45	29.35	.779	28.0
TADG		25.34	24.91	25.03	25.01	.917	15.0
ADF-g/day							
ADF1	7	30.52	32.69	32.92	32.92	.129	10.0
ADF2	14	43.10	43.67	43.81	42.73	.786	8.1
ADF3	21	66.24	65.02	70.09	66.49	.752	9.8
ADF4	28	71.86	71.22	73.12	72.49	.675	10.4
TADF		52.71	53.30	54.82	53.27	.422	6.0
FD/gain	7	1.45	1.49	1.44	1.44	.362	5.3
FD/gain	14	2.31	2.43	2.55	2.53	.351	14.8
FD/gain	21	2.05	2.16	2.24	2.14	.579	16.0
FD/gain	28	2.49	2.42	2.57	2.47	.745	14.0
TFD/gain		2.08	2.14	2.19	2.13	.336	6.8

[a,b]Means in rows with different superscripts differ (P<.05).

TABLE 25. Effect of Nickel Level on Hematology (Experiment 2)

Item	Units	Nickel Level (ppm) 0	10	50	100	P Value	CV
Hct	% RBC	28.46[b]	28.45[b]	27.36[a]	28.78[b]	.006	10.7
Hb	g/100ml	8.60[b]	8.78[b]	8.62[b]	9.20[a]	.007	15.0
Plasma-SSA (mg/kg)							
Nickel		.168[b]	.170[b]	.290[a]	.261[a]	.001	13.4
Iron		.943	.919	.938	.955	.906	12.7
Copper		.077	.081[b]	.081[b]	.089[a]	.022	11.6
Zinc		1.643	1.636	1.774	1.688	.603	15.6
Plasma-TCA (mg/kg)							
Nickel		.374[b]	.355[b]	.368[b]	.421[a]	.001	20.2
Iron		1.469	1.495	1.472	1.384	.103	21.8
Copper		.105[b]	.116[a]	.119[a]	.100[b]	.001	31.7
Zinc		1.817[b]	1.646[a]	1.767[b]	1.747[b]	.024	22.2

[a,b]Means in rows with different superscripts differ (P<.05).

concentrations but there was a trend toward decreasing gain as nickel concentration increased at day 21. ADG at day 28 and total ADG were not significantly affected by nickel concentration. Average daily feed intake was not significantly affected by dietary nickel concentration.

Feed:gain was not significantly affected by added dietary concentrations of nickel at any time period during the experiment. Hematocrit was significantly lower ($P<.05$) for those chicks fed added dietary nickel at 50 ppm compared to those chicks fed added dietary nickel at 0, 10, and 100 ppm. Hemoglobin content was significantly higher for those animals fed added dietary nickel at 100 ppm. Sulfosalicylic acid treated plasma exhibited a significantly higher ($P<.05$) nickel concentration for those animals fed added dietary nickel at 50 and 100 ppm. SSA treated plasma had a significantly higher ($P<.05$) copper concentration for those chicks fed added nickel at 100 ppm. Iron and zinc concentrations of SSA treated plasma were not significantly affected by added dietary concentrations of nickel. Trichloroacetic acid treated plasma had a significantly higher ($P<.05$) nickel concentration for added dietary nickel concentrations of 100 ppm than for plasma from birds on 0, 10, and 50 ppm added nickel. Copper concentration for TCA treated plasma was significantly higher ($P<.05$) for animals fed added dietary nickel concentrations of 10 and 50 ppm but not for those animals fed added nickel concentrations of 0 and 100 ppm. Zinc concentration for TCA treated plasma was significantly lower ($P<.05$) for chicks fed added nickel concentration at 10 ppm but not for chicks fed added nickel

concentrations of 0, 50, and 100 ppm. Iron concentration was not significantly affected by added dietary concentrations of nickel for TCA treated blood plasma.

Data illustrating the effect of added dietary concentrations of nickel on tissue mineral concentrations are presented in Tables 26 and 27. Nickel concentration in heart tissue, calculated on a wet basis, was significantly higher ($P<.05$) for those chicks on diets with added dietary nickel concentration of 100 ppm. Iron concentration of heart tissue, on a wet basis, was significantly higher ($P<.05$) for chicks on added concentrations of nickel at 100 ppm but not for those chicks fed added dietary concentrations of nickel at 0, 10, and 50 ppm. Zinc concentration of heart tissue, on a wet basis, was significantly higher ($P<.05$) for chicks on added dietary concentrations of nickel at 100 ppm. Copper concentration of heart tissue was not significantly affected by added dietary concentrations of nickel. When expressed on a dry basis no significant differences in heart mineral content were obtained but zinc tissue concentrations approached significance ($P<.08$) on a dry basis. Nickel concentration of kidney tissue was significantly higher ($P<.05$) for those chicks fed the added dietary concentration of nickel at 100 ppm, on a dry matter basis, but not for those chicks fed added concentrations at 0, 10, and 50 ppm nickel. Iron, copper, and zinc concentration of kidney tissue were not significantly affected by added dietary concentrations of nickel.

TABLE 26. Effect of Nickel Level on Heart Tissue Mineral Levels (mg/kg) (Experiment 2)

Heart	Nickel Level (ppm) 0	10	50	100	P Value	CV
Nickel Wet	.67[b]	.74[b]	.70[b]	.82[a]	.015	42.1
Nickel Dry	4.32	3.61	3.58	4.13	.543	95.5
Iron Wet	19.81[b]	22.40[b]	22.26[b]	23.19[a]	.028	34.7
Iron Dry	104.70	116.58	115.03	113.45	.311	38.7
Copper Wet	2.00	2.13	2.41	2.52	.086	69.5
Copper Dry	11.15	10.99	11.28	11.85	.603	35.0
Zinc Wet	8.80	9.15	8.75	9.77	.012	24.0
Zinc Dry	46.47	47.41	46.23	48.73	.694	27.9

[a,b]Means in rows with different superscripts differ (P<.05)

TABLE 27. Effect of Nickel Level on Kidney Tissue Mineral Levels (mg/kg) (Experiment 2)

Kidney	Nickel Level (ppm) 0	10	50	100	Value	CV
Nickel Wet	.05	.06	.10	.15	.144	89.0
Nickel Dry	.28[b]	.30[b]	.44[b]	.51[a]	.032	54.8
Iron Wet	4.84	3.90	5.30	6.03	.469	65.8
Iron Dry	22.55	18.31	23.09	20.65	.586	47.1
Copper Wet	.30	.25	.48	.29	.337	92.6
Copper Dry	1.40	1.19	1.48	1.33	.719	53.2
Zinc Wet	1.57	1.43	1.69	1.55	.784	47.4
Zinc Dry	7.23	6.62	7.99	7.17	.752	50.0

[a,b]Means in rows with different superscripts differ (P<.05).

CHAPTER V

DISCUSSION

I. Introduction

The effects on performance and physiological parameters of feeding various concentrations of nickel in the diets of swine and chicks are contradictory and often confusing. Weber and Reid (1968) reported that dietary concentrations of 100 and 300 ppm nickel, in either the carbonate or acetate form, had no adverse effect when fed to chicks from hatching to 4 weeks of age. Ling and Leach (1979) in a study with chicks, reported no interaction between nickel, iron, copper, and zinc. When nickel was fed at toxic concentrations the addition of the other four minerals singularly, or in combination, did not negate the symptoms of nickel toxicity. The lack of interaction between nickel, iron, copper, and zinc is in contrast to the results observed by Nielsen (1980b) in a recent literature review that indicated that nickel interacts with at least 13 minerals in animal and plant systems. Kirchessner and Roth (1977) reported a study where nickel was observed to have had a toxic effect on young pigs. Nickel supplemented at a concentration of 375 ppm as nickel sulfate caused a slight depression in gain (5%) and feed intake (3%). Nickel supplementation of 500 ppm significantly reduced gain (8%) and feed intake (6%). O'Dell et al., (1970) found that nickel chloride was more toxic than nickel carbonate when fed at concentrations ranging from 100 to 1000 ppm. Most studies that have been conducted with nickel in animals were either at very low concentrations of

added dietary concentrations of nickel, ranging from 1 to 30 ppm, or with relatively high added dietary concentrations of nickel, ranging from 100 to 1300 ppm. For this reason we decided to investigate an intermediate range of 0, 10, 50, and 100 ppm added nickel. The possible interaction of nickel and iron, with respect to iron form and iron concentration was also investigated. Compared to the other trace metals that are recognized as being nutritionally essential, there has been very little research conducted with respect to nickel effects on performance.

II. Experiment 1 - Performance

In experiment I there was a trend for decreased gain with ferric iron as compared to experimental treatments utilizing ferrous iron. This type of response is somewhat similar to that observed in rats (Nielsen, 1980c). There was a trend for feed intake to decrease as added nickel concentration increased. Whether this trend was due to a decrease in palatability or due to a toxic effect of added nickel concentrations was impossible to ascertain in this experiment.

Nickel added at 50 ppm decreased daily gain during the second, third and fourth week of the experiment. The effect of added nickel at this concentration was cubic in nature with gain decreasing as nickel concentration increased until at 50 ppm there was a significant decrease in gain, and at 100 ppm added nickel gain increased so that it was not significantly different from added concentrations of 0 and 10 ppm nickel.

The interaction between nickel concentration and iron form during the second half of the experiment was significant. Pigs receiving ferric iron gained less at added concentrations of 50 ppm nickel while pigs receiving ferrous iron at 50 ppm added nickel had gains which were similar to the other three concentrations of nickel. This resulted in a significant nickel concentration by iron form interaction.

III. Experiment 1 - Hematology

Red cell concentration was not affected by form of iron but nickel added at 50 ppm increased RBC concentration at week four of the experiment. White blood cell concentrations were not affected by added nickel concentrations or form of iron. This could imply that nickel's effect on RBC concentration is not acute and may be a secondary effect due to an as yet unknown primary toxic affect. The fact that WBC concentration in this experiment was not affected by nickel concentration seems to imply that nickel does not have a role in white cell mobilization, at least in the short term. Hematocrit was higher for nickel added at 50 ppm during the last week of the experiment but not for the first 3 weeks of the experiment. There was an interaction between nickel at 50 ppm and ferric iron at day 28. Hematocrit was higher for nickel at 50 ppm and ferric iron as compared to nickel at 50 ppm and ferrous iron.

There was a tendency for hemoglobin to be increased at higher concentrations of nickel when pigs received ferric iron but not to as great an extent with ferrous iron.

Nickel, iron, copper, and zinc concentrations in hair were not significantly affected by nickel concentration or iron form but there was a clear trend for iron concentration in hair to be higher for added dietary concentrations of nickel at 50 ppm and for iron concentrations to be higher for nickel at 50 ppm with ferric iron as compared to ferrous iron. The results of this hair study would lead one to conclude that hair is not a good indicator of nickel status in the animal.

In experiment 1 blood plasma was treated in two different manners. One of the questions asked in this experiment was whether nickel in blood plasma was protein bound or free. To this end the plasma sample from each individual animal was split and one half treated with sulfosalicylic acid in order to precipitate the intact proteins and concurrently any nickel bound to these proteins. The other half of the plasma was treated with trichloroacetic acid in order to destroy the intact proteins and so liberate nickel bound therein.

Added nickel concentrations of 100 ppm nickel increased nickel concentrations of SSA treated plasma at day 21 but not for the remainder of the experiment. In experiment 1 this is one of only two instances where added nickel at 100 ppm had a significant effect on a dependent varible. This may imply that, in this experiment, nickel treatment effect on blood concentration was decreased at 100 ppm as compared to dietary concentrations of 50 ppm added nickel possibly because nickel at 100 ppm is not being absorbed or utilized as readily as nickel fed at 50 ppm.

Dietary nickel concentrations of 50 ppm and 100 ppm increased iron concentrations of SSA treated plasma over nickel concentrations of 0 and 10 ppm for the last two weeks of the experiment for nickel at 50 ppm, and higher than 10 ppm at days 21 and 28, and higher than 0 at day 28 for 100 ppm added nickel. This is only the second instance where nickel at 100 ppm had a significant effect on a dependent varible. Centrifugation of sulfosalicylic acid treated plasma results in the protein fraction of the plasma being removed along with any nickel that may be protein bound. This results in a smaller total amount of nickel in blood plasma and may give the appearance that nickel at 100 ppm has more significance than is actually the case due to the very small concentrations being measured. Ferric iron significantly increased copper concentrations across all concentrations of added nickel during the last two weeks of the experiment as compared to ferrous iron. This suggest the possibility that ferric iron may either enhance copper absorption or nickel absorption which in turn may affect copper absorption. Zinc concentration of SSA treated plasma was not significantly affected by form or concentration of added iron although added nickel tended to decrease plasma zinc concentrations by a small increment.

Nickel concentration in trichloroacetic acid treated plasma was not significantly affected by form of iron or nickel concentration for the entire experiment but ferric iron increased iron concentration of TCA treated plasma for days 21 and 28 across all concentrations of nickel. Copper concentration was higher for TCA treated plasma with nickel added at 50 ppm for days 14, 21, and 28

and ferric iron as compared to nickel at 50 ppm and ferrous iron. Zinc concentration was not affected by form of iron or added nickel concentrations.

In analyzing the data from experiment 1, several points appear evident. In the young pig nickel does not appear to be readily absorbed or concentrated to a high degree in the various tissues studied, this is in apparent agreement with earlier studies conducted by Kirchgessner and Schnegg (1980) and (Schroeder et al., 1974). Added dietary nickel concentrations of 50 ppm had a growth suppression effect that was not evidenced at higher concentrations of dietary nickel (100 ppm). Blood tissue concentrations of nickel and iron also were increased with nickel added at 50 ppm as where hematocrit and RBC concentration of blood. This suggest several possibilities. At 100 ppm added nickel the absorptive capabilities of the gut may be impaired (J. Moore, unpublished data) resulting in less nickel absorption than when nickel is fed at 50 ppm so that the systemic effects of nickel absorption are no longer evident. The absorption of nickel is generally believed to be primarily an energy driven process. With 100 ppm added nickel in the diet, a homeostatic control mechanism may be activated that reduces the absorption of nickel by the mucosa. Kirchgessner and Roth (1977) reported that 500 ppm added nickel depressed rate of gain in swine. At these comparatively high amounts of added nickel the homeostatic control mechanisms could be exceeded. At very high concentrations Ni may be absorbed by passive diffusion. Ferric iron seems to act in a synergistic manner with nickel as compared to ferrous iron. The

effects of nickel seem to be enhanced when combined with the ferric form of iron. It is possible that since nickel in the chloride form, as used in this experiment, is divalent, that ferrous iron and nickel^{+2} may compete for binding sites in the mucosa during the absorption process. Becker et al., (1980) reported that nickel absorption was increased during iron deficiency, suggesting a common intestinal transport system. As with divalent nickel, ferric iron absorption is considered to be an energy driven process. This suggest the possibility that in the young pig nickel may actually function as a structural component or cofactor in specific metalloenzymes or as a bioligand cofactor to facilitate the intestinal absorption of the ferric iron. From the data presented it appears that hair is not a good indicator of short term nickel status in the animal as there were no significant differences in hair concentrations of Ni, Fe, Cu, and zinc regardless of dietary nickel concentration or iron form. Hair concentrations of nickel did tend to increase at days 21 and 28 as compared to the first two weeks of the experiment. The plasma study with either TCA or SSA treated plasma would seem to suggest that nickel is bound to a plasma protein and that only approximately 30% of the nickel found in plasma is in the free state. When total nickel concentration is required TCA treated plasma would appear to be the method of choice for analysis of nickel in blood plasma.

IV. Experiment 2 - Performance

On day 14 ferrous iron supplemented chicks had a higher average daily gain than did those on the ferric iron supplemented diets, but on day 28 those chicks on ferric iron supplemented diets had a higher average daily gain. Total average daily gain was not affected by form of iron. The inconsistency with respect to form of iron could possibly be the result of iron oxide being introduced into the experiment from waterers that may have contained some iron oxide in small amounts of rust, it has been established that chicks can utilize iron oxide as a nutritional source of iron.

Chicks fed diets supplemented with ferrous iron had a significantly higher average daily feed intake (ADF) than those supplemented with ferric iron, for days 7 and 14, but not for days 21 and 28 nor for total ADF for the experiment. The data suggest that ferrous iron may improve growth and feed intake during the very early growth period of chicks but is of less importance as the chick ages. On day 14 ADG was significantly decreased by nickel added at 100 ppm. There was a trend toward decreased ADG as nickel concentrations increased at day 14. It appears that ADG was decreased linearly as nickel concentrations increased in the diet suggesting a growth suppression effect for added concentrations of nickel in the chick within the range used.

V. Experiment 2 - Hematology

Hemoglobin was not affected by dietary concentration of iron, but hemoglobin content was significantly higher for chicks

supplemented with ferric iron compared to ferrous iron supplemented diets. Copper concentration in SSA treated plasma was higher for ferric iron treated diets than for ferrous iron supplemented diets. It appears that ferric iron supplemented diets were congruent to higher hemoglobin concentration and copper concentration in SSA treated plasma. Ferrous iron seemed to promote increased nickel concentration and iron concentrations in TCA treated plasma. This could possibly be because of plasma-free and ferrous iron protein bound in the blood plasma.

Hemoglobin content of blood was higher for those animals fed added dietary nickel at 100 ppm. The means for both 50 and 100 ppm added nickel were such that it appears that nickel concentration response is very nearly a linear relationship in the chick for hemoglobin.

Sulfosalicylic acid and TCA treated plasma exhibited a higher nickel concentration for those animals on added dietary concentrations of 50 and 100 ppm nickel. Iron concentration of both SSA and TCA treated plasma was not affected by added concentrations of nickel in experiment 2, but copper concentration was significantly higher for those chicks fed added nickel at 100 ppm for SSA treated plasma but not for plasma treated with TCA. This would suggest the possibility that there is a higher concentration of free copper (non-protein bound) in blood plasma at higher dietary nickel concentrations as compared to protein bound copper. When plasma is treated with TCA in order to liberate all bound nickel the difference

could be masked by the comparitively large amount of copper released into the plasma.

VI. Experiment 2 - Tissue Concentrations

Iron form and iron concentration did not have a significant effect on nickel, iron, copper, and zinc concentrations of kidney and heart tissue on a wet or dry basis. There appeared to be a trend for higher concentration of kidney and heart tissue concentrations of nickel, iron, copper and zinc for diets supplemented with ferric iron compared to diets supplemented with ferrous iron. This trend suggest the possibility that ferric iron is more compatible or that ferrous iron is more antagonistic to absorption and/or utilization of these metals. There was a trend for nickel supplemented diets at 50 ppm to have a higher concentration of iron, copper, and zinc for kidney tissue calculated on both a wet and dry basis compared to added dietary concentrations of 0, 10 and 100 ppm. This more approaches the values for various tissues examined in experiment 1. The primary difference between the two experiments, in terms of added concentrations of nickel, appeared to be that the responses due to nickel supplementation were in a more linearly oriented fashion than in experiment 1, which had a more cubic response. This difference could be due to species differences between pigs and chicks in that different transport proteins, methods of absorption, homeostatic control mechanisms, and enzyme systems may have functioned under the conditions of these experiments.

CHAPTER VI

SUMMARY

Possible physiological interactions between nickel and iron were investigated in chicks and weaned baby pigs with special emphasis on the effect of form and concentration of iron on nickel and their effects on performance, hematology and mineral parameters.

In the first experiment 48 Yorkshire X Duroc X Hampshire weaned pigs from 8 litters and averaging 9.8 kg were housed individually in 76 X 60 cm expanded metal pens. They were divided into 8 groups and fed diets containing 0, 10, 50 or 100 parts per million (ppm) added nickel (Ni) as the chloride and 60 ppm iron (Fe) as ferrous or ferric sulfate in a 4 X 2 factorial arrangement. Feed mixed with water as a gruel was offered twice daily to appetite and additional water was to satiation after each feeding. Pigs were weighed and blood was sampled weekly to obtain performance, hematological data and plasma minerals (Ni, Fe, Cu and Zn). Hair was clipped from the right shoulder and hip at 14, 21 and 28 days for mineral analysis.

Pigs fed Ni added at 50 ppm to the basal diet had lower ($P<.05$) average daily gain (ADG) after 14 days and required more ($P<.05$) feed per unit gain (F/G) than did pigs fed the other Ni concentrations. Pigs receiving ferric Fe also required more F/G than did those given ferrous Fe. Although not affected significantly by form of Fe across all concentrations of Ni, ADG was decreased with ferric but not with ferrous Fe at 50 ppm added Ni. Addition of 50 ppm Ni increased ($P<.05$) red cell concentration, hematocrit and hemoglobin at 28 days

and concentrations of Ni, Fe and Cu in pigs fed 50 ppm added Ni were higher when ferric as compared with ferrous Fe was fed. Dietary concentration of nickel and form of Fe had no effect on mineral content of hair.

Addition of 50 ppm Ni decreased performance and increased hematological and plasma minerals in pigs. Nickel chloride appeared to interact in a synergistic manner with ferric sulfate but not with ferrous sulfate on the parameters studied.

In the second experiment 384 male broiler chicks of the Hubbard X Hubbard strain were fasted overnight, weighed to the nearest gram, wing banded and allotted to 48 treatment groups with similar average initial weights. Groups were assigned to 48 wire bottomed cages within two batteries containing 24 cages each. Three replicates per treatment with eight birds per replicate were randomly allotted to 0, 10, 50 or 100 ppm added nickel from nickel chloride, each with 60 or 100 ppm Fe as ferrous sulfate in a 4 X 2 X 2 factorial arrangement. The birds were fed and watered ad libitum for the 4 weeks of the experiment. They were weighed at the start of the experiment and thereafter at weekly intervals. At the termination of the experiment all chicks were bled via heart puncture and 12 tissues were obtained from each bird for analysis.

Average daily feed intake (ADF) and ADG were higher ($P<.05$) for chicks fed 60 ppm as compared with 100 ppm Fe for the first 2 weeks of the experiment. However, at day 21 ADG was higher ($P<.05$) when Fe was supplemented at 100 ppm rather than at 60 ppm. On day 28 ADG was higher ($P<.05$) for chicks fed diets supplemented with ferric Fe

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rather than for those fed ferrous Fe supplemented diets. However, ADF was significantly higher for chicks fed diets supplemented with ferrous Fe than when ferric Fe was fed. Feed:gain ratio was not affected significantly by either form or concentration of Fe.

Hematocrit was affected by neither form nor concentration of Fe. Although not affected by concentration of Fe, hemoglobin concentration was higher ($P<.05$) when chicks were supplemented with ferric rather than ferrous Fe. Nickel concentration in trichloroacetic acid treated plasma was increased ($P<.05$) for chicks fed diets supplemented with ferrous Fe. Plasma Fe concentration was higher ($P<.05$) for chicks fed 60 rather than 100 ppm Fe and higher ($P<.05$) when ferrous rather than ferric Fe was added.

Average daily gain was increased ($P<.05$) on day 7 by Ni added at 50 and 100 ppm, was decreased ($P<.05$) on days 14 and 21 by 100 ppm added Ni, but at day 28, ADG and total ADG were not significantly affected by Ni concentration. Neither ADF nor feed:gain were significantly affected by added dietary Ni. Added Ni decreased ($P<.05$) hematocrit at 50 ppm but increased ($P<.05$) hemoglobin concentration at 100 ppm. Plasma Ni concentration was increased ($P<.05$) by added dietary Ni at 50 and 100 ppm when plasma was treated with sulfosalicyclic acid and at 100 ppm when plasma was treated with TCA. Nickel concentration was higher when plasma was treated with TCA than when plasma was treated with SSA. Concentrations (wet basis) of Ni and Fe were higher ($P<.05$) in kidney tissue in chicks fed 100 ppm added Ni.

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