THESIS

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CLINICAL SIGNS, PATHOLOGY AND SUSCEPTIBILITY OF ZINC-DEFICIENT CALVES TO ENTERIC INFECTION

By

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ABSTRACT

CLINICAL SIGNS, PATHOLOGY AND SUSCEPTIBILITY OF ZINC-DEFICIENT CALVES TO ENTERIC INFECTION

By

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Six, 6-month-old calves were used to determine the clinical signs and pathology of zinc deficiency and their susceptibility to an enteric infection. All 6 calves were fed a basal, low zinc ration for 50 days. Four of the 6 calves were injected with different amounts of $CaNa_2EDTA$ and 2 of these 4 were orally supplemented with zinc. At the end of 50 days of these treatments, all calves were exposed to *E. coli* and coronavirus and necropsied 5 days later.

The clinical signs associated with zinc deficiency were inappetence, apathy and skin changes. These changes consisted of alopecia, scaliness and bleeding fissures of the skin, especially on the legs. Serum and hepatic zinc values were lowest in calves injected with $CaNa_2EDTA$ and not supplemented with zinc. Microscopically, there was a depletion of lymphocytes in the thymuses of calves with low serum and hepatic zinc values. Exposure to *E. coli*-coronavirus produced a diarrhea in 3 of the 6 calves. In 2 of these 3 calves with diarrhea, serum zinc, albumin and total protein values were decreased. Histologically, the intestinal villi of calves with diarrhea were denuded of epithelium or covered by a cuboidal type of epithelial cells.

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Zinc-deficient calves were susceptible to enteric infection and the resulting diarrhea decreased serum and hepatic zinc concentrations.

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INTRODUCTION

Since the original report on the requirement of zinc for the rat by Todd, Elvehjem and Hart in 1934 and the findings of Tucker and Salmon in 1955 that the disease parakeratosis of swine was caused by a zinc deficiency, this nutrient has been the object of intensive research. The zinc requirements of several species have been determined and, although a great deal is known about the absorption, intermediary metabolism and excretion of zinc, many questions remain as to its role in immunological processes and resistance to infection.

Natural and experimental zinc deficiency has been described in cattle. Skin changes, decreased feed efficiency and retarded growth are the main features of the deficiency. A hereditary zinc deficiency with typical skin lesions, hypoplasia of lymphoid tissues and immunological disturbances occurs in cattle and has been suggested as an animal model for research on acrodermatitis enteropathica in man.

Increased susceptibility to infections, decreased cell-mediated immunity (CMI) and hypoplasia of lymphoid tissues, especially the thymus, have been reported in zinc-deficient animals and in chronically ill human patients with low concentration of zinc in serum. The administration of zinc to these patients or animals partially restores thymic weight and CMI and increases resistance to infections. Increased susceptibility to infections, decreased CMI and hypoplasia of lymphoid tissues also occur in protein-calorie malnutrition (PCM) in man. Interestingly, low concentrations of zinc in serum are reported in at

least some cases of PCM, and an increase in thymic size has been documented after zinc administration.

The metabolism of zinc is altered by specific diseases. A low molecular weight protein (leukocytic endogenous mediator - LEM) liberated by leukocytes during infection is believed to promote zinc redistribution in the organism during pathological states. Infections, especially of the digestive tract, contribute to malnutrition by disturbing the digestion of food and absorption of nutrients. Malnutrition, in turn, predisposes to infections by debilitating the organism and interfering with defense mechanisms. This combination of infection and malnutrition is a major cause of calf mortality and an important economic problem in cattle-raising countries of the world. *Escherichia coli* and the coronavirus are frequently the causative agents of enteritis in calves and several other domestic species.

A number of factors that interfere with zinc absorption and metabolism have been identified. The more important ones are high calcium rations and the presence of phytic acid in cereal feeds. Calcium and phytate are capable of inducing zinc deficiency in pigs under practical feeding conditions.

Calcium disodium ethylenediaminetetraacetate ($CaNa_2EDTA$) is widely used in the treatment of lead intoxication in man. Calcium disodium EDTA chelates lead and increases its urinary excretion. It also chelates zinc and increases urinary excretion of zinc.

Additional information on the role of zinc in calf nutrition and in the resistance to infection by the young calf would greatly enhance healthy and profitable cattle production in many countries of the world.

In undertaking this research on zinc deficiency in calves, the emphasis on the pathology of the skin was used by Dr. C. Lopes in his

research. The emphasis on $CaNa_2EDTA$ was in cooperation with Dr. J. Spaulding.

REVIEW OF LITERATURE

General

A voluminous amount of general information is available on the role of zinc in nutrition and health of man and animals. This review pertains primarily to the role of zinc in cattle nutrition and health.

Zinc was first demonstrated to be an essential nutrient for animals in 1934, when Todd, Elvehjem and Hart, using a purified type of ration, produced a zinc deficiency in the rat. The deficiency was characterized by reduced weight gain and a loss of hair around the head, shoulder and ventral parts of the body. This demonstration of the essentiality of zinc was possible because of an extensive purification of all diet components to remove zinc and the extreme care in housing and handling of the rats in order to avoid any contact with extraneous sources of zinc. These facts, coupled with the presence of relatively high amounts of zinc in plant foods and mineral supplements, led nutritionists to conclude that zinc deficiency would never be of practical importance in livestock (Miller, 1970). Nevertheless, a disease of unknown etiology that affected young swine in drylot feeding conditions since 1942 was described by Kernkamp and Ferrin in 1953. This disease was characterized by the presence of keratinous crusts on the surface of the skin, mostly over the legs and head, and microscopically by parakeratosis, the term proposed by the authors as a provisional name for the disease. Tucker and Salmon (1955), while

comparing hydraulic and solvent extracted peanut meal as a protein source for swine in 1950, observed a severe dermatitis concomitant with impaired growth. Other clinical signs were diarrhea, vomiting, anorexia and loss of weight. Death occurred in severe cases. In reviewing the literature, these researchers concluded that the occurrence of the disease was widespread. Searching for the cause of the disease, these researchers demonstrated that parakeratosis of swine was a manifestation of zinc deficiency and was precipitated by a high level of calcium and/or phosphorus in the ration and a relatively high requirement for zinc by the pig.

O'Dell and Savage (1957) reported the beneficial effect of zinc supplementation on weight and bone growth of chicks fed a "Drackett" processed soy-protein ration containing 50 ppm of zinc. In the same experiment zinc had no beneficial effect when added to a casein or "alpha" soy-protein ration having no more zinc than the "Drackett" soy-protein ration. The authors concluded that zinc from "Drackett" soy-protein might be unavailable to the chick. Experimental zinc deficiency has been produced and described in several species, among them cattle (Miller and Miller, 1960; Ott et al., 1965; Mills et al., 1967), goats (Miller et al., 1964), sheep (Ott et al., 1964; Mills et al., 1967), dogs (Robertson and Burns, 1963), pigs (Tucker and Salmon, 1955; Lewis et al., 1957; Miller et al., 1968), chickens (Edwards et al., 1958; Young et al., 1958) and laboratory animals (Todd et al., 1934; Hove et al., 1937; Fraker et al., 1977). Naturally occurring zinc deficiency in man has been reported in young males in Iran and Egypt (Prasad, 1979a). Hambidge et al. (1972) reported poor growth, anorexia and hypogeusia in children in Colorado. These children also had low zinc values in the hair. Zinc supplements normalized taste

acuity and increased hair zinc values. The authors speculated that the clinical signs observed could be related to a zinc deficiency.

Decreased appetite, poor growth and skin lesions are constant findings in experimental and spontaneous zinc deficiency. The mechanism by which zinc deficiency brings about these changes is not known. It is known, however, that zinc is a component of approximately 70 different enzymes (Prasad, 1979a), and it has been hypothesized that the clinical signs and lesions seen in zinc deficiency are a consequence of enzymatic changes in the organism (Miller, 1970).

Zinc is present in several dehydrogenases, aldolases, peptidases and phosphatases required in a variety of metabolic processes. Alkaline phosphatase, carbonic anhydrase, carboxypeptidases A and B, and alcohol dehydrogenase, among others, have been reported to be decreased during zinc deficiency (Prasad, 1979b).

Zinc may have its primary effect on zinc-dependent enzymes that regulate the synthesis and catabolism of nucleic acids (Prasad, 1979b). Increased ribonuclease activity in zinc-deficient tissues was found by Prasad and Oberleas in 1973, and evidence for decreased activity of deoxythymidine kinase during zinc deficiency was found by the same authors in 1974 (Prasad, 1979a). In some of the enzyme molecules, zinc participates in catalysis and also appears to be essential for the maintenance of the structure of apoenzymes (Prasad, 1979b).

Naturally Occurring Zinc Deficiency in Cattle

Legg and Sears (1960) reported a seasonal disease of range cattle in British Guiana with lesions closely resembling those of rats fed a zinc-deficient ration. The disease was characterized by parakeratosis of the muzzle, vulva, anus, top of tail, ears, back of hind legs, "knee fold", flanks and neck. In the more acute cases lesions spread over 40%

of the body surface. Body condition and growth of affected cattle were poor. Rapid recovery was observed after weekly treatments with 2 grams of zinc sulfate orally or 1 gram by injection. Haaranen (1962) described an itching eczema in the tail root of young cattle and dry milking cows in Finland. The daily oral administration of 800 mg of zinc chloride resulted in cessation of itching within 3 to 5 days, and 240 mg of zinc oxide had the same effect within 6 to 14 days. A recurrence was observed 14 days after ZnO administration was discontinued. Spontaneous recovery was observed in cows after the start of lactation. The serum and urine concentrations of calcium were higher in affected than in nonaffected cattle. The opposite was observed for magnesium. Serum alkaline phosphatase activity was low in sick animals. Based upon the beneficial effect of the zinc therapy and studies on the relationship between calcium and zinc levels in the feeds, Haaranen (1963) concluded that this disease was caused by an absolute or relative zinc deficiency. In subsequent studies it was reported that "itch, hair licking, alopecia and general unthriftiness occur at low feed zinc or copper levels", indicating the participation of copper as well as zinc in the pathogenesis of the disease (Haaranen, 1965).

Dynna and Havre (1963) reported a complex zinc-copper deficiency in young cattle in Norway, characterized by unthriftiness, depigmentation of the hair around the eyes and sometimes a grayish thickening of the skin with loss of hair on the nose. The administration of zinc aggravated the clinical signs, whereas copper administration resulted in partial recovery. When both minerals were given, total recovery occurred. Histopathological studies did not reveal parakeratosis.

Demertzis and Mills (1973) reported the beneficial effects of zinc in the control of infectious pododermatitis in young bulls without clinical signs of zinc deficiency and stated that under some conditions a suboptimal zinc status may escape recognition because of the absence of specific clinical signs in these conditions.

Hereditary Zinc Deficiency in Cattle

A hereditary disease of cattle characterized by parakeratosis, alopecia, failure to grow, hypoplasia of lymphoid tissues and an increased susceptibility to infections has been identified in several European countries (Brummerstedt et al., 1977). This disease affects Friesian cattle and is transmitted by an autosomal recessive gene. Calves are born apparently normal and clinical signs appear 4 to 8 weeks after birth. The calves usually die because of secondary infections before 4 months of age or are otherwise slaughtered because of their poor condition (Andresen et al., 1970). Changes in the lymphoid tissues include hypoplasia of the thymus, spleen, some regional lymph nodes, Peyer's patches and lymphoid tissue along the intestine. The thymus is histologically depleted of small lymphocytes, especially in the cortical region (Brummerstedt et al., 1977). Immunological studies revealed a decreased humoral immune response to tetanus toxoid and a decreased cell-mediated immunity against *Mycobacterium tuberculosis* and dinitrochlorobenzene (Brummerstedt et al., 1974). This disease is similar to zinc deficiency in cattle (Brummerstedt et al., 1977) and zinc supplementation leads to complete recovery, including restoration of thymic morphology (Brummerstedt et al., 1971). Zinc absorption was found to be impaired in animals affected with the trait (Flagstad, 1976). Hereditary zinc deficiency in cattle closely resembles acrodermatitis

enteropathica in man and has been suggested as an animal model for experimental studies (Weismann and Flagstad, 1976; Brummerstedt et al., 1977).

Experimental Zinc Deficiency in Cattle

Experimental zinc deficiency in cattle was first produced by Miller and Miller (1960, 1962) at the University of Georgia using egg-white protein diets containing 2.7 and 3.6 ppm of zinc. Excessive salivation, reduced feed intake and weight gain, a dull and listless appearance, undersized testicles, alopecia, dermatitis, bowing of the hind legs and swelling of the feet and joints were observed in deficient calves. The skin changes were more pronounced in the legs, head and lower parts of the body and histologically consisted of parakeratosis. Other integumentary changes were stomatitis, horny overgrowths on the mucosa of lips and dental pads, and overgrowth of the ruminal papillae with moderate keratin formation. Parakeratosis of the ruminal wall and esophageal mucosa was present in deficient and control calves. Lack of roughage was pointed out as the possible cause for the ruminal parakeratosis. The zinc content of blood and various tissues was lower in the deficient calves. Blood carbonic anhydrase activity was decreased in zinc-deficient calves and correlated with whole blood zinc values.

Ott et al. (1965) at Purdue University confirmed most of the findings of Miller and Miller (1960, 1962) and did extensive histological studies in zinc-deficient calves. Contrary to the findings of Miller and Miller (1960, 1962), these authors found hyperkeratosis rather than parakeratosis to be the main histological difference in the skin of control and deficient calves. No differences were found in the oral mucosa, esophagus, rumen, reticulum, omasum, abomasum, small intestine,

large intestine, kidney, liver, spleen, pancreas, thyroid, hypophysis, adrenal, brain and spinal cord of control and deficient animals. Deficient calves had an elevated rumen pH, with an overall reduction in the concentration of acids. Reduced feed consumption and feed efficiency were also observed in the deficient calves. Feed efficiency improved with zinc supplementation. Biochemical changes in zinc deficiency were: slightly reduced blood glucose values, increased serum proteins, with gamma globulin fractions having the greatest increases, and low serum zinc concentrations.

Mills et al. (1967) in Scotland confirmed the gross findings of the previous workers when determining the zinc requirements of calves and lambs. It is interesting to note that, like Ott et al. (1965) at Purdue, the skin lesions are characterized as hyperkeratosis rather than parakeratosis.

Zinc Requirement of Cattle

Zinc deficiency in calves has been produced by feeding rations containing from 0.9 to 6 ppm zinc (Miller and Miller, 1960, 1962; Ott et al., 1965; Miller et al., 1965a; Miller et al., 1966; Mills et al., 1967). Mills et al. (1967) in Scotland reported that 8 ppm of zinc in the ration was adequate to maintain a good rate of growth in calves, but 10 to 14 ppm were required to maintain normal plasma zinc concentrations. Their findings were in accordance with the suggestion of Miller et al. (1963) that 9 ppm of zinc should be sufficient to meet the growth requirements of calves.

Still, some contradiction exists as to the zinc requirement of cattle. Skin diseases responsive to zinc therapy where the zinc contents of feedstuffs were 18 to 42 ppm (Legg and Sears, 1960), 45 ppm (Haaranen,

1965) and 19 to 83 ppm (Dynna and Havre, 1963) have been reported.

Demertzis and Mills (1973) reported the beneficial effects of zinc administration on the recovery of bulls suffering from infectious pododermatitis where the feed provided 30 to 56 ppm of zinc. In their study, zinc supplementation improved weight gains in diseased but not in healthy bulls fed the same ration. Since pododermatitis did not occur in steers or in heifers under the same conditions, the authors suggested that bulls might have a higher zinc requirement.

Perry et al. (1968) at Purdue University obtained more rapid gains in feedlot cattle in 2 experiments when zinc was added to rations containing 24 to 29 ppm of zinc, but in 2 other trials zinc supplements had no effect.

Zinc supplements were reported to increase milk production of dairy cows fed a ration of corn silage and concentrate having 13 and 43 ppm of zinc, respectively (Voelker et al., 1969). On the other hand, performance of dairy cows fed a practical ration containing 44 ppm of zinc over a 6-week period was not improved by zinc administration (Miller et al., 1965b), no were milk production, feed intake or body weight of first lactation Holstein cows fed a ration containing 17 ppm of zinc over the same period of time (Neathery et al., 1973). In the latter research, however, the cows adjusted to the amount of zinc in the ration, as reflected by decreased milk zinc and increased zinc absorption by the cows fed 17 ppm as compared to controls fed 40 ppm of zinc. Studies by Stake et al. (1973) indicated that with the more rapid growth observed with practical rations as compared to purified rations, the zinc requirements of calves fed practical rations probably would be higher.

Although there are no studies on the zinc requirements of bulls, it is interesting to note that Underwood and Sommers (1969) found 17 ppm of zinc to be adequate for the growth of lambs, but testicular growth and sperm production were improved when 32 ppm were fed. In a review article on the zinc nutrition of cattle, Miller (1970) wrote:

> In most instances, the factors which may affect the [zinc] requirements of ruminants are unknown. Differences in availability or absorption may be influenced either by the chemical form and association of the zinc or by other constituents of the diet.

The National Research Council (1976, 1978) recommendations for zinc in rations are 20 to 30 ppm for beef and 40 ppm for dairy cattle.

Effects of Zinc Deficiency on the Lymphoid System and Immunity

Thymic hypoplasia during experimental zinc deficiency has been reported to occur in the pig (Miller et al., 1968; Whitenack et al., 1978), the rat (Quarterman, 1974) and the mouse (Fraker et al., 1977). Hypoplasia of the thymus and other lymphoid tissues has been found in cattle with hereditary zinc deficiency (Andresen et al., 1970; Brummerstedt et al., 1971; Weismann and Flagstad, 1976; Brummerstedt et al., 1977) and in man with acrodermatitis enteropathica, conditions in which zinc absorption is known to be impaired (Weismann and Flagstad, 1976).

Depletion of lymphocytes in the cortical areas of the thymus has been described as a constant feature in calves with hereditary zinc deficiency (Brummerstedt et al., 1977). The same changes were described by Whitenack et al. (1978) in the zinc-deficient pig.

Immunological studies in cattle with hereditary zinc deficiency indicated decreased cell-mediated immunity against *Mycobacterium tuberculosis* and dinitrochlorobenzene (Brummerstedt et al., 1974).

Decreased cell-mediated immunity also occurred in zinc-deficient mice (Fraker et al., 1977) and rats (Pekarek et al., 1977).

Atrophy of the thymus and lymph nodes as well as decreased cell-mediated immunity have been described in protein-calorie malnutrition (Neumann et al., 1975). Golden et al. (1977) found reduced thymic size in children with protein-calorie malnutrition. Low serum zinc values were also recorded in these children and zinc supplementation promoted thymic growth, as verified radiologically.

Increased susceptibility to infections, as reflected by high incidence of diarrhea, conjunctivitis, rhinitis, stomatitis and bronchopneumonia, was reported in calves with hereditary zinc deficiency (Brummerstedt et al., 1971; Brummerstedt et al., 1977). Increased susceptibility to infections has also been reported as a constant feature of acrodermatitis enteropathica in man (Weismann and Flagstad, 1976).

Zinc Metabolism During Pathological Processes

Zinc metabolism is known to be altered during diseased states. Halsted and Smith Jr. (1970) reported decreased plasma zinc values in patients with active tuberculosis, indolent ulcers, uremia, myocardial infarction, nontuberculous pulmonary infections, Down's syndrome, cystic fibrosis with growth retardation, alcoholic cirrhosis and other liver diseases. Lowered plasma zinc concentrations were present in patients with psoriasis, venous leg ulcerations and several dermatoses (Greaves and Boyde, 1967).

Recently, Whitenack et al. (1978) reported the influence of transmissible gastroenteritis (TGE) infection on the zinc metabolism of piglets. In this study, TGE infection lowered the serum zinc

concentrations of pigs fed a zinc-adequate diet but had no effect on the serum zinc concentrations of zinc-deficient pigs. The enteric infection also consistently decreased zinc retention in zinc-deficient pigs. Furthermore, TGE aggravated the clinical signs and lesions and increased mortality due to zinc deficiency.

Leukocytic endogenous mediator (LEM), a low molecular weight protein liberated by leukocytes and present in the plasma of infected animals, has been emphasized as the promoter of reduced plasma zinc concentrations during pathological states (Pekarek and Beisel, 1971). Low plasma albumin values found during diseased states have also been attributed to the action of LEM (Powanda, 1979). Leukocytic endogenous mediator induced significant alterations in the zinc metabolism of the rat, stimulating the uptake of this metal by the liver (Pekarek et al., 1972).

Coronaviral Diarrhea of Calves

Coronaviruses are pathogenic to several species, including man (Tyrrel et al., 1978). Coronaviral diarrhea of young calves is known to occur since 1972, even though the definite classification of the agent as a coronavirus was accomplished only in 1976 (Sharpee et al., 1976). Coronavirus is one of the most important agents of calf diarrheas (England, 1977). In a recent study it was found in 16.4% of diarrheic calves 1 day to 1 year old (Langpap et al., 1979). In the latter study, coronavirus was found to infect calves up to 3 months of age. Coronavirus has also been isolated from healthy adult cattle (England, 1977) and calves (England, 1977; Morin et al., 1978).

Mebus et al. (1973) reported the incubation period of coronaviral diarrhea after oral inoculation to be around 20 hours. Elimination of

yellowish liquid feces was the typical clinical sign. Death of infected calves occurred within 48 to 62 hours after the onset of diarrhea. Dehydration was usually present and fever did not always occur. Increased hematocrit, plasma protein, plasma potassium concentration and plasma osmolarity, as well as decreased blood pH, were reported with coronavirus-induced diarrhea (Lewis and Phillips, 1978).

Shortening of villi, especially in the distal parts of the small intestine and colon, has been described as the most constant microscopic lesion of coronavirus diarrhea in calves (Mebus et al., 1975). Microscopically, besides this change, fusion of adjacent villi, lining of the villi with cuboidal or almost squamous epithelial cells, and infiltration of the submucosa by lymphocytes, plasma cells, macrophages and sometimes neutrophils and eosinophils have been found (Mebus et al., 1975; Langpap et al., 1979).

Escherichia coli Diarrhea of Calves

Colibacillosis is a common disease of newborn farm animals. Enterotoxic colibacillosis is the most common form of colibacillosis in newborn calves. It affects calves from 1 day to 3 weeks of age (Blood et al., 1979), although it is more frequent in calves less than 4 days old (Morin et al., 1978).

Recently, Bellamy and Acres (1979) studied the pathology of enterotoxigenic colibacillosis in colostrum-fed calves orally exposed to 3.5×10^{11} live *E. coli*. A profuse yellowish diarrhea was observed 6 to 8 hours after inoculation. Other clinical signs were dehydration and weakness. The gross findings were dehydration, petechiae in the abomasal mucosa and watery yellow contents of localized areas of the small intestine, cecum and colon. Microscopically, stunted, shortened villi

covered by cuboidal epithelium, a layer of gram-negative bacteria and focal accumulations of neutrophils above the dome areas of the Peyer's patches were in the jejunum and ileum. This combination of microscopic lesions was thought to be specific for *E. coli*. Similar lesions were found by the authors in 2 naturally infected calves. Stunted villi covered by cuboidal epithelium were also reported by Pearson et al. (1978) in colostrum-deprived calves orally exposed to 5 to 20 x 10^9 *E. coli* and protected from colisepticemia by systemic administration of immunoglobulin M.

Toxicity of Ethylenediaminetetraacetic Acid (EDTA) Salts

Ethylenediaminetetraacetate and its sodium salt have been used as industrial and analytical reagents due to their property of chelating divalent and trivalent metals. Calcium disodium ethylenediaminetetraacetate ($CaNa_2EDTA$) has its pharmacological use today in the treatment of lead poisoning based upon the high affinity of this metal for the compound and its ability to displace calcium from the chelate (Levine, 1975).

Perry and Perry (1959) studied the effects of intravenous administration of $CaNa_2EDTA$ on the urinary concentration of zinc and other trace metals in human subjects. Calcium disodium EDTA increased the urinary excretion of zinc by 10-fold. A smaller increase occurred in the excretion of cadmium and manganese.

The nephrotoxic action of EDTA and its salts is well known. Dudley et al. (1955) reported severe renal damage with necrosis of the epithelial cells of the proximal convoluted tubules in 2 hypercalcemic patients given large amounts of sodium EDTA. Vacuolation of the epithelium and, to a lesser extent, necrosis were also present in the distal

tubules. It is interesting to note that small splenic malpighian bodies and decreased lymphocytic population in the lymph nodes were present in both patients.

Foreman et al. (1956) reported toxic tubular nephrosis in a woman undergoing treatment with $CaNa_2EDTA$ and the occurrence of the same lesion in rats given varying amounts of the drug. They demonstrated that $CaNa_2EDTA$ caused severe hydropic degeneration of the proximal tubules and that this lesion regressed in a few days after administration was discontinued. Furthermore, the daily dose required to produce histological evidence of renal injury in 50% of the rats over a period of 16 days was 203 mg per kg of body weight (mg/kg BW). No lesions could be demonstrated when the daily dose was 62.5 mg/kg BW over the same period of time. Tubular nephrosis in rats given EDTA salts has been confirmed by other researchers (Altman et al., 1962; Doolan et al., 1967). Ahrens and Aronson (1971) reported necrotizing nephrosis of the proximal convoluted tubules and necrosis of intestinal epithelium with loss of villous structure in dogs intravenously infused with $CaNa_2EDTA$.

The maximum recommended daily dose of $CaNa_2EDTA$ for the treatment of lead intoxication in man is 50 mg/kg BW and the treatment should not exceed 5 days (Levine, 1975).

Summary

A large volume of information emphasizes the dynamic and important role of zinc in maintaining optimum health in man and animals. Much of the available information pertains to the role of zinc in nutrition, its chemistry and metabolism. Only a meager amount of information is available on the role of zinc in resistance to natural infection and

immunologic processes. Reports on the role of zinc in experimental infection in cattle were not found in the literature. Additional experimental work in livestock would seem of great importance to determine the role of zinc in promoting efficient and profitable animal production.

OBJECTIVES

The objectives of this research were to determine in young calves the following:

1. The clinical signs and pathology of zinc deficiency. Special emphasis was given to lesions in the lymphoid tissues.

2. The susceptibility of malnourished, zinc-deficient calves to an *E. coli* and coronavirus enteric infection.

3. The effect of *E. coli* and coronavirus diarrhea on serum zinc, total protein and albumin values.

4. The influence of calcium disodium ethylenediaminetetraacetate injections on the concentrations of zinc in the liver and the serum.

MATERIALS AND METHODS

General

The calves used in this research were originally in a group of 12 Holstein calves obtained from the Michigan State University Dairy Herd in the fall of 1977. They were a few days of age when obtained and weighed an average of 53 kg. They were maintained in 2 rooms in Building F at the Veterinary Research Farm. During the first month the calves were fed whole milk which was gradually replaced with a low zinc basal ration using egg-white as the low zinc protein. The initial design was to feed 8 calves the zinc-deficient ration and 4 calves the same ration supplemented orally with 25 mg zinc (sulfate) 3 times a week. At approximately 1 month of age, milk feeding was discontinued and the zinc-deficient ration was increased. The increased amount of the egg-white zinc-deficient ration was associated with a decreased performance in the calves. This was manifested by inappetence, diarrhea and an unthrifty appearance of the calves. After considerable feeding trials, it was finally concluded that these young calves could not tolerate a large amount of egg-white as a source of protein. At 5 months of age the calves were all fed ration 2, which contained mainly soy-assay rather than egg-white as the protein. Of the 12 original calves, 6 calves either died due to complications associated with the egg-white protein or were unthrifty and not suitable for further research.

Experimental Design, Housing and Care

The 6 calves used in this research were approximately 6 months of age and weighed an average of 57 kg. They were maintained in individual wooden pens, with concrete floors partially covered with rubber mats. The pens were washed daily. Daily records were maintained on each calf as to clinical signs, food consumption and general health. Weight changes were recorded weekly. The total duration of the experiment was 55 days (i.e., 50 days for the study of zinc deficiency and 5 days for the study on the interaction of a zinc deficiency and an enteric infection).

Of the 6 calves, 2 (4 and 9) were fed the basal ration (ration 2). One calf (8) was fed ration 2 and injected daily with 18.5 mg of $CaNa_2EDTA$/kg BW, subcutaneously. Another calf (11) was fed ration 2 and injected daily with 37 mg of $CaNa_2EDTA$/kg BW, subcutaneously. The last 2 calves (10 and 12) were fed ration 2, given 50 mg of zinc (sulfate) orally in gelatin capsules 3 times a week, and injected subcutaneously with 37 mg of $CaNa_2EDTA$/kg BW, daily. The experimental design is summarized in Table 2 (page 37).

Experimental Rations

The composition of the rations is given in Table 1.

The initial ration (ration 1) contained 6 ppm zinc and was a modification for calves of the ration used previously in zinc deficiency research in rats and mice by Luecke et al. (1968). While rats and mice were able to utilize egg-white protein as a protein with a low content of zinc, calves could not. Ration 2 contained 8 ppm zinc and, while not as low in zinc as ration 1, it was suitable for this research. The low-zinc mineral mixture was prepared by Dr. E. R. Miller and was of the

Table 1. Diet composition

Ingredients	Ration 1*	Ration 2*
Dried egg white[a]	1800	300
Isolated soybean protein[b]	---	1000
Low zinc, mineral mixture[c]	350	400
Cellulose[d]	300	300
Ground corn cobs[e]	2000	2000
Glucose monohydrate[f]	4450	4820
Complete vitamin mixture[g]	100	100
Fat (lard)	1000	1000
Urea (48% N)	---	80
Oxytetracycline hydrochloride[h]	5	5
Total	10,005	10,005

*Amount of component in grams.

[a]Egg-white solids, Teklad Test Diets, Madison, WI.

[b]Soya assay protein, Teklad Test Diets, Madison, WI.

[c]Mineral mixture kindly provided by Dr. E. R. Miller and according to Miller et al. (1968).

[d]Cellulose, Solka Floc, Brown Co., Chicago, IL.

[e]Bed-o'cobs, Andersons Cob Division, Maumee, OH.

[f]Cerelose, Corn Products Co., Argo, IL.

[g]Vitamin fortification mix, Teklad Test Diets, Madison, WI.

[h]Terramycin soluble powder, Pfizer, Agricultural Division, New York, NY.

same composition as the one used previously in extensive studies of zinc deficiency in the young pig (Miller et al., 1968).

Calcium Disodium EDTA Injections

Calcium disodium EDTA was injected subcutaneously in the form of a 10% sterile solution in normal saline, mixed with 50% its volume of 2% lidocaine.[a] Lidocaine was injected with the $CaNa_2EDTA$-saline mixture to avoid pain produced by the EDTA salts.

Experimental Infection

The calves were exposed to *E. coli* and coronavirus 50 days after the start of feeding basal ration 2. The coronavirus used for exposure of the calves was obtained from Dr. L. E. Newman and was infective in his previous research (Newman et al., 1978). It was maintained at -70 C until immediately prior to inoculation. The *E. coli* was isolated from the intestine of a 3-day-old diarrheic calf necropsied at the Animal Health Diagnostic Laboratory and identified in Dr. G. R. Carter's laboratory. To expose the calves, 10 ml of the coronaviral inoculum and 28 ml of a heart-brain infusion *E. coli* culture containing 9×10^8 bacteria/ml as determined in the McFarland nephelometer (Frankel et al., 1970) were used. The coronavirus-*E. coli* inoculum was added to approximately a pint of milk and given to each calf in a nipple bottle.

Starting at 2 days prior to exposure of the calves to infection, the antibiotic was not included in the ration.

The temperature of the calves was recorded each morning from 2 days before exposure until the end of the experiment. During this same

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[a]Lidocaine hydrochloride injection, U.S.P. 2%, D-M Pharmaceuticals, Inc., Rockville, MD.

time detailed clinical signs were recorded at frequent intervals between 8 a.m. and 5 p.m. and, when appropriate, later in the evening.

Serum Collection

Serum for protein and mineral analyses was collected every other day during the week before the coronavirus-*E. coli* exposure and daily after the exposure. Prior to the above period, samples were collected weekly. The blood samples were collected in plastic disposable tubes, allowed to clot at room temperature, then centrifuged and the serum separated using disposable glass Pasteur pipettes and placed into 5 ml plastic disposable tubes. The tubes were covered with a double layer of parafilm and kept at -20 C until analyzed.

Histopathology and Tissue Collection

The calves were killed and necropsied 5 days after exposure to *E. coli* and coronavirus. Tissues for histopathology were collected and fixed in 10% buffered formalin. Liver samples for zinc analyses were placed in plastic bags and stored at -20 C until analyzed. Lung, liver, kidney, ileum and colon were aseptically collected for microbiologic examination. Intestinal samples were kept in a separate container. Tissues for histopathologic examination were processed according to routine techniques and stained with hematoxylin and eosin (Luna, 1968).

Laboratory Procedures

Serum, liver and ration samples were analyzed using an atomic absorption spectrophotometer.[b] Serum zinc was determined according to Dubowski (1971). The zinc content of the ration and the liver and serum

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[b]Perkin-Elmer 360, Perkin-Elmer, Norwalk, CT.

copper, magnesium and iron were determined according to procedures recommended by the manufacturer of the atomic absorption spectrophotometer, except that for the zinc determinations the standard solutions recommended by Dubowski (1971) were used to calibrate the instrument.

Total serum protein was measured with a Goldberg's refractometer.[c] Serum protein electrophoresis was performed according to routine technique (Golias, 1976).

Packed cell volume, hemoglobin concentration and white blood cell counts were done manually according to standard laboratory techniques.

Routine bacteriological examination was conducted in lung, liver, kidney, ileum, and colon samples. Fluorescent antibody technique for coronavirus presence was conducted on ileum and colon, essentially as described by Reed et al. (1971).

Statistical Analyses

The data were analyzed by the one-sided Student's t-test whenever useful to their interpretation.

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[c]National Photometer, National Instrument Co., Inc., Baltimore, MD.

RESULTS

The results will be given as to (1) the clinical signs observed during the initial 50 days before the exposure to infection and the gross and microscopic lesions attributable to zinc deficiency, (2) the effects of $CaNa_2EDTA$ on serum and liver zinc concentration and the pathology of the kidneys, (3) the clinical signs, clinical pathology and pathology of intestine following exposure to *E. coli* and coronavirus and (4) the data on serum zinc, total protein and albumin concentrations during the enteric infection.

Clinical Signs

The calves did not have any clinical signs of zinc deficiency during the first 10 days. During this period food consumption was about the same for each calf and was approximately 1 kg/calf/day. All the calves were alert and active, frequently moving around in the pens, running, jumping or kicking especially at the time of the morning feeding. The alert and playful behavior of the animals was also manifested by the licking and bumping of individuals working inside the pens during the morning washing of the floor. Usually the calves laid down and got up several times a day. Most usually they did get up if they were down when people entered the rooms. Pica, manifested by frequent licking of each other, wood chewing and the chewing of strings and ropes used to tie the water buckets to the walls of the pens, was observed in all calves. This behavior was characterized by eating of small amounts of

ration alternating with chewing of the boards on the wall of the pens above the feeder. The pica started shortly after the calves were fed the dry ration, when still a few weeks old, and continued to the end of the experiment even after the exposure to *E. coli* and coronavirus.

The first clinical signs of zinc deficiency appeared at approximately 10 days in the calf given 37 mg of $CaNa_2EDTA$/kg BW (treatment 3). These signs were decreased food intake and less active behavior. By day 15, food consumption of this calf was decreased by about 30% of its previous consumption. It also developed apathy, increased sensitivity of the feet and skin lesions. It had a dull appearance, being very quiet and with the eyes apparently fixed on an imaginary object in the air. The hair coat was rough and without its previous shiny character. Scaliness of the skin was observed and was more pronounced in the ventral surface of the neck and jaw and around the mouth, muzzle and eyes. The hair on the limbs distal to the carpal and tarsal joints was matted. This change was very pronounced in the hair of the feet, immediately above the coronary band. Increased sensitivity of the feet was reflected by a "stepping" gait with constant shifting of the weight to different limbs and slight alternating raising of one foot or another from the floor. The calf had difficulty in lying down and when down was unwilling to get up. Palpation of the feet was painful, as evidenced by attempts to pull the foot being palpated. In contrast, none of the other calves had any of the described clinical signs at that time. Their appetite remained the same, they were active, the hair coat was smooth and shiny, and no increased sensitivity of the feet was noticed. The total daily food intake of the calf having clinical signs of zinc deficiency slowly returned to the amount observed during the first 10 days of the experiment. By day 23 the food intake was the same

as the daily intake observed during the first 10 days, but the calf was slower in eating as compared to the others, eating a smaller amount of food but more frequently. This slow eating pattern, as well as the other described clinical signs, remained essentially the same for the rest of the experiment. By the end of the experiment the skin changes in the calf given 37 mg $CaNa_2EDTA$/kg BW were severe. Several areas of alopecia with fissures and bleeding of the skin were present on the limbs. These areas varied in size and shape, most of them being almost circular, 2 to 5 cm in diameter and located on the lateral aspect of the limbs. These lesions were more numerous in the hind legs. There was matting of the hair, alopecia and crusts of blood and debris on the feet (Figures 1 and 2) and hock (Figure 3). Scaliness and areas of alopecia were present on the thighs (Figure 4). Palpation of the skin of the neck, thorax and abdomen of this calf (11) revealed it to be thicker and less pliable than the skin of the other calves. The other calves appeared to be normal throughout the experiment.

The average weight gain for each calf was 16 kg for the entire 55-day period. No differences were detected at any time in the weight gain of any of the calves.

Gross Pathology

All the calves were emaciated. The average body weight at the time they were necropsied was 75 kg. Decreased thymic size was observed in all calves, but it was particularly prominent in the calf given 37 mg $CaNa_2EDTA$/kg BW without zinc supplementation. Thymic weights varied from 20 to 53 g, averaging 41 g for the 6 calves. Thymic weights relative to body weight are given in Table 2.

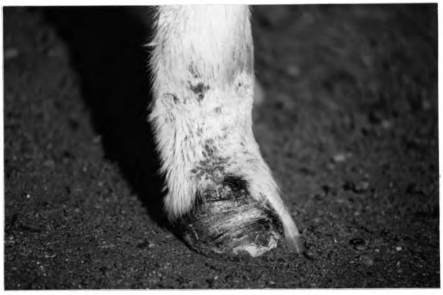

Figure 1. Matting of hair and crusts of debris on the left front foot of calf 11, fed a ration containing 8 ppm zinc and injected daily with 37 mg $CaNa_2EDTA$/kg BW.

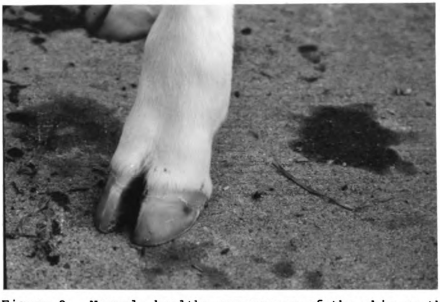

Figure 2. Normal, healthy appearance of the skin on the left rear foot of calf 12, fed a ration containing 8 ppm zinc, injected daily with 37 mg $CaNa_2EDTA$/kg BW and given 50 mg of zinc 3 times a week.

Figure 3. Matting of hair and a crust of dried blood and debris on the left hock of calf 11, fed a ration containing 8 ppm zinc and injected daily with 37 mg $CaNa_2EDTA$/kg BW.

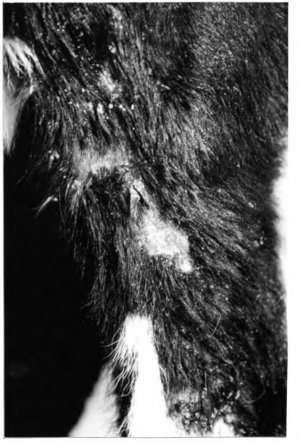

Figure 4. Scaliness and alopecia on the left thigh of calf 11, fed a ration containing 8 ppm zinc and injected daily with 37 mg $CaNa_2EDTA$/kg BW.

Lesions not likely related to zinc deficiency were: overgrowth of the ruminal papillae, abomasitis, 1 ulcer and several erosions on the abomasum, and pneumonia. The overgrowth of the ruminal papillae was present in all calves and was characterized by long papillae that were fused together forming plaques. This change was more pronounced in the dorsal sac of the rumen. The abomasitis was present in 1 calf (4). This lesion consisted of a thick mucus deposit covering an area of approximately 5 cm in diameter located in the fundic area of the abomasum. Several strands of hair perforated the mucosa. Two hairballs approximately 5 cm in diameter were found in the rumen of this calf. The abomasal erosions were in 3 calves (8,9,10). These consisted of 3 to 5 depressions approximately 0.2 cm in diameter in the mucosa of the fundus, with little evidence of inflammation around them. The ulcer was present in calf 12. It was about 1 cm in diameter and located in the cardiac region.

The pneumonia consisted of several hard nodules, 0.3 to 0.5 cm in diameter, in the lung of calf 12. These nodules were mostly located in the vicinity of the bronchiolar tree. When compressed, a yellowish-white viscid fluid exuded from the nodules.

Microscopic Pathology

Depletion of lymphocytes in the cortical areas of the thymic lobules was observed in the 2 calves given $CaNa_2EDTA$ without zinc supplementation. The cortex of these lobules was thin and there was a concomitant increase in the size of the medulla (Figure 5). The thymic lobules of the calves supplemented with zinc had a much larger population of lymphocytes, with thicker cortices and smaller medullas (Figure 6).

Figure 5. Depletion of lymphocytes in the cortical area of the thymic lobules of calf 11, fed a ration containing 8 ppm zinc and injected daily with 37 mg $CaNa_2EDTA$/kg BW. Notice the narrow cortices (arrows) and wide medullas (M). Hematoxylin and eosin stain; X65.

Figure 6. Normal population of lymphocytes in the cortical area of the thymic lobules of calf 12, fed a ration containing 8 ppm zinc, injected daily with 37 mg $CaNa_2EDTA$/kg BW and given 50 mg of zinc 3 times a week. Notice the wide cortices (arrows) and narrow medullas (M). Hematoxylin and eosin stain; X65.

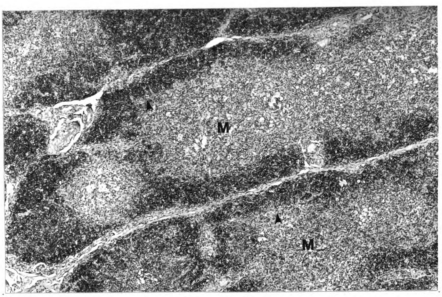

Figure 5

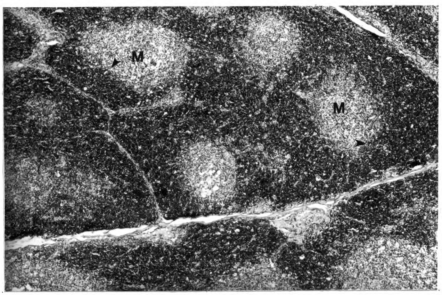

Figure 6

Severe parakeratosis was present in several areas of the skin of calf 11, given 37 mg $CaNa_2EDTA$/kg BW (treatment 3). This lesion was characterized by the retention of nuclei within the stratum corneum of the epidermis and acanthosis.

The overgrowth of the ruminal papillae seen grossly in all calves was characterized microscopically by parakeratosis. This lesion consisted of a thickening of the stratum corneum of the epithelium with retention of elongated pyknotic nuclei. Acanthosis was also present. Parakeratosis was also present in the esophagus and tongue of all calves.

The abomasitis in calf 4 was characterized microscopically by necrosis of the mucosal surface. The mucosa was covered by a large amount of mucus and contained pieces of hair surrounded by areas of necrosis heavily infiltrated by neutrophils. Neutrophils were also present in the lamina propria around the necrotic areas. The abomasal erosions seen grossly in 3 calves consisted of necrosis of the upper half of the mucosa and a mild neutrophilic infiltration of the lamina propria around these areas.

The ulcer in the abomasum of calf 12 was a large hemorrhagic area covered with tissue debris and infiltrated by a large number of neutrophils. The necrosis covered the whole depth of the mucosa. The submucosa was edematous and infiltrated by neutrophils. Granulation tissue was present under the ulcer and extended to the muscularis. Mineral deposits were present along the limiting line between the mucosal debris and the inflamed submucosa.

The pneumonia seen grossly in calf 12 consisted of several areas of peribronchiolar consolidation. Some of these areas were heavily infiltrated by inflammatory cells, predominantly macrophages. In other areas the inflammatory cells were mostly neutrophils. Bronchiolar

dilation, hyperplasia of the mucosa with focal areas of squamous metaplasia, lymphoid hyperplasia and collections of inflammatory cells within the bronchiolar lumina were also present.

No lesions or differences could be found in any of the other organs examined, including salivary gland (parotid), pancreas, liver, gallbladder, trachea, urinary bladder, skeletal and cardiac muscle, aorta, testicle, lymph nodes, pituitary, thyroid, and adrenal.

Effects of $CaNa_2EDTA$

The serum and hepatic zinc concentrations are given in Table 2. Serum zinc values in calf 11, fed the deficient ration and given 37 mg $CaNa_2EDTA$/kg BW (treatment 3) ranged from 17 to 29 µg/dl with an average of 24 µg/dl for the entire 50 days of the experiment. The serum zinc concentration was 17 µg/dl by day 15, when clinical signs of zinc deficiency were observed. Calf 8, given a smaller amount of $CaNa_2EDTA$ (treatment 2), had serum zinc values ranging from 19 to 67 µg/dl and averaged 35 µg/dl during the entire experiment. During the last 30 days of the experiment, the highest serum zinc concentration of calf 8 was 35 µg/dl. The calves fed just the basal ration (treatment 1) had serum zinc concentrations from 35 to 83 µg/dl with an average of 54 µg/dl were observed in calves given $CaNa_2EDTA$ (37 mg/kg BW) and zinc supplements. For all the calves, the higher serum zinc values were most generally observed in the initial period of the experiment.

Liver zinc concentrations were higher in calves not given $CaNa_2EDTA$ (treatment 1). Among the calves given EDTA, zinc-supplemented calves had slightly higher hepatic zinc concentrations.

No lesions that could be attributed to the action of $CaNa_2EDTA$ were observed in the kidneys. Grossly the kidneys of calf 4, not given

Table 2. Summary of serum and liver zinc values, thymus weight and thymic lymphocytic population in calves fed basal ration and calves fed basal ration and given $CaNa_2EDTA$ with or without zinc supplementation

Treatment	No. Calves	Zinc Values Serum* (µg/dl)	Zinc Values Liver (ppm)	Thymus Wt/BW (g/kg)	Thymic Lymphocytic Population
(1) Basal Ration (8 ppm Zn)	2(4&9)	54±14	53&26	.883 &.862	normal
(2) Basal Ration + 18.5 mg EDTA/kg SQ daily	1(8)	35±15	21	.611	depleted
(3) Basal Ration + 37 mg EDTA/kg SQ daily	1(11)	24±04	22	.363	depleted
(4) Basal Ration + 37 mg EDTA/kg SQ daily + 150 mg Zn/wk orally	2(10& 12)	41±11	25&24	.426 &.850	normal

*Mean ± standard deviation of 10 to 13 samples from each calf, collected before the exposure to *E. coli* and coronavirus.

Statistical significance:

Serum zinc values:

Treatment (1) is different from treatments (2) and (3), $p<.0005$.
Treatment (2) is different from treatment (4), $p<.005$.
Treatment (2) is different from treatment (3), $p<.025$.
Treatment (2) is different from treatment (4), $p<.10$.
Treatment (3) is different from treatment (4), $p<.0005$.

Liver zinc values and thymus weight:

Treatment (1) is different from treatment (4), $p<.20$.

$CaNa_2EDTA$, had an even appearance without discernible differences between cortex and medulla on cut section. Microscopically, there were mineralization of epithelial cells of the tubules, collections of neutrophils within the tubular lumina, and scattered neutrophils around the tubular basement membrane.

Experimental Enteric Infection

Clinical Signs

A profuse watery diarrhea occurred in 3 calves (9,11,12) 7 hours after the oral inoculation with coronavirus and *E. coli*. The feces were greenish in color and had streaks of yellowish mucus. At that time the rectal temperature increased from a previous average of 102.2 F to 104.8 F. The calves stopped eating and were depressed. Clinical examination revealed a slight dehydration. During the second day after exposure, the 3 calves kept passing fluid feces and had a high temperature and anorexia. Forty-eight hours after the inoculation, the average temperature of these calves was 103.3 F. No feces were found in the stalls of these calves on the third day. Appetite returned to normal. On the fourth day all the 3 previously mentioned calves (9,11,12) eliminated pasty feces but had a normal temperature and appetite. No abnormalities were observed on the fifth day. Calves 4, 8 and 10 remained apparently normal throughout the experiment.

Clinical Pathology

No consistent changes were detected in packed cell volume, hemoglobin concentration or total and differential white cell counts in any of the calves.

Necropsy Findings

The only gross abnormality observed during necropsy was a subserosal edema in the spiral colon of calf 4.

Histopathology

Histologic lesions in the intestine consisted of short, broad villi that were denuded of epithelium or covered by cuboidal or flat epithelial cells (Figures 7 and 8). Occasional collections of neutrophils were in the lumen of the mucosal glands. A few neutrophils were also at the basement membrane on the tip of the villi. These changes were severe and generalized in the jejunum and ileum of calves 4, 9, 11 and 12. The same but milder lesions were in the colon. In contrast, only occasional shortening and denuding of the villi were in the intestinal sections of calves 8 and 10. In these calves the intestinal villi were covered by tall cylindrical epithelial cells with abundant cytoplasm (Figures 9 and 10). Numerous goblet cells were in the epithelium. Large numbers of lymphocytes and eosinophils were in the lamina propria and submucosa of the intestine of all calves. In the same way, very active lymphoid tissue was in the ileal sections of all calves.

Bacteriology

Light growth of *E. coli* was obtained from the intestine of all calves.

Virology

Results of examination for fluorescent antibody in the intestine of calves 4, 9, 11 and 12 were positive for coronavirus.

Figure 7. Short villi covered by cuboidal epithelial cells (arrow) in the ileum of calf 9, fed a ration containing 8 ppm zinc and having diarrhea after exposure to 2.5 x 10^{10} *E. coli* and coronavirus. Hematoxylin and eosin stain; X160.

Figure 8. Low cuboidal epithelial cells (arrow) covering the villi of the ileum of calf 9, fed a ration containing 8 ppm zinc and having diarrhea after exposure to 2.5 x 10^{10} *E. coli* and coronavirus. Notice inflammatory cells (i) in the lamina propria. Hematoxylin and eosin stain; X400.

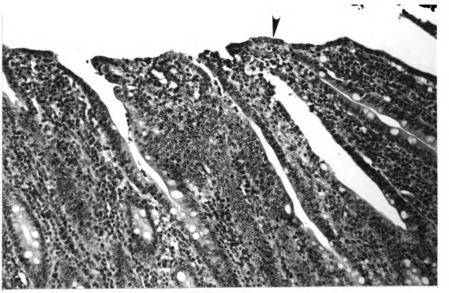

Figure 7

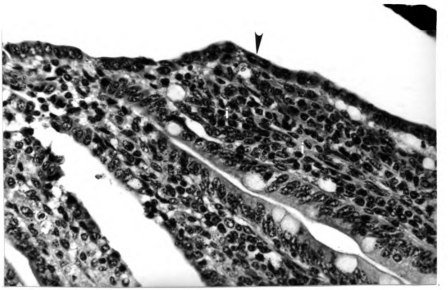

Figure 8

Figure 9. Long, slender villi covered by tall columnar epithelial cells (arrow) in the ileum of calf 10, fed a ration containing 8 ppm zinc, injected daily with 37 mg $CaNa_2EDTA$/kg BW, given 50 mg of zinc 3 times a week and remaining normal after exposure to 2.5 x 10^{10} *E. coli* and coronavirus. Hematoxylin and eosin stain; X160.

Figure 10. Tall cuboidal epithelial cells (arrow) covering the villi of the ileum of calf 10, fed a ration containing 8 ppm zinc, injected daily with 37 mg $CaNa_2EDTA$/kg BW, given 50 mg of zinc 3 times a week and remaining normal after exposure to 2.5 x 10^{10} *E. coli* and coronavirus. Notice inflammatory cells in the lamina propria. Hematoxylin and eosin stain; X400.

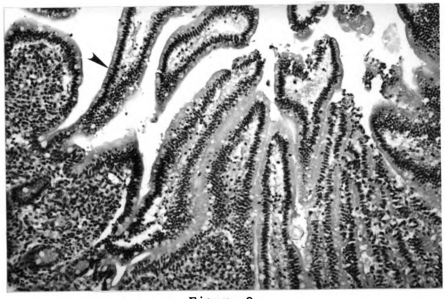

Figure 9

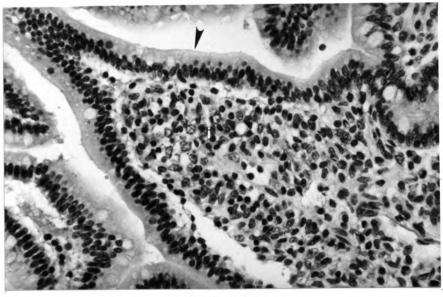

Figure 10

Effect of Enteric Infection on Serum Zinc and Proteins

Data on the serum zinc, total protein and albumin concentrations are given in Table 3. Serum zinc values were significantly lower after the experimental infection in the 3 calves with severe diarrhea (9,11,12). In 2 of these calves (9 and 11) serum total protein and albumin were also lowered after the infection. These 2 parameters were also lowered in one calf (4) without diarrhea but positive for coronavirus. Serum total protein and albumin were elevated in calf 8 after infection.

Table 3. Summary of the influence of diarrhea on the serum zinc, total protein and albumin concentrations

Clinical Response and Virology	Calf No.	Serum Zn (µg/dl) Before*	After**	P	Serum TP (g/dl) Before***	After**	P	Serum Albumin (g/dl) Before***	After**	P
No diarrhea; FA negative for coronavirus	8	35±15	23±03	NS	5.1±.14	5.1±.12	.10	2.30±.14	2.53±.06	.05
	10	42±10	45±14	NS	5.1±.14	5.1±.12	NS	2.60± 0	2.67±.15	NS
No diarrhea; FA positive for coronavirus	4	55±14	61±11	NS	6.1±.14	5.6± 0	.005	2.55±.07	2.27±.21	.10
Diarrhea; FA positive for coronavirus	9	52±14	22±08	.005	5.0± 0	3.9±.26	.01	2.65±.07	1.83±.23	.01
	11	24±04	15±03	.005	5.5±.14	5.0±.20	.05	2.60± 0	2.23±.15	.025
	12	40±12	22±08	.025	5.0± 0	4.7±.31	NS	2.55±.07	2.37±.23	NS

*Mean ± SD of 10 to 13 samples collected before the exposure to *E. coli*-coronavirus.

**Mean ± SD of 3 samples collected 24, 48 and 72 hours after the exposure to *E. coli*-coronavirus.

***Mean ± SD of 2 samples collected 24 and 48 hours before the exposure to *E. coli*-coronavirus.

DISCUSSION

This research on the interrelationship between zinc deficiency and infection in cattle was somewhat frustrating, laborious and time consuming. However, it did supply information and training as to how nutritional-infection problems might exist in malnourished herds such as those found in underdeveloped countries. The research illustrated that specific enteric infections have an influence on nutritional requirements of zinc.

In general, the research suggested that the zinc requirements of cattle were altered by a variety of factors, such as the type of ration, feed consumption, rate of growth and infectious agents. It was a disappointment that so much of a problem was precipitated by including the egg-white as a source of low zinc protein. Previous researchers did not mention this problem. However, in most instances their experimental animals were at a slightly older age when their research was initiated (Miller and Miller, 1960; Ott et al., 1965; Mills et al., 1967). The problem encountered suggested that in the young calf the gastrointestinal tract might in some way be very sensitive to egg-white protein. Since egg-white is cited to be a rather complete protein, it seems that additional research to determine its digestibility and deleterious effects to young calves would be most useful. This was not the objective of this research.

The ration used in this experiment was not as low in zinc as was originally planned. Because of this, it took a longer time for the

clinical signs of zinc deficiency to develop and they were not as severe as reported by previous researchers. It was thought, however, that the conditions in this experiment more closely resembled the conditions in malnourished herds.

Studies combining nutrition and infection in cattle are very difficult to undertake. However, in practical cattle production these problems are not isolated and thus the results are more realistic. Devising palatable rations deficient in a single factor and that are otherwise nutritionally adequate for large animals is expensive. Superimposing infectious agents that will produce reliable clinical signs, lesions and specific immunologic reactions is also difficult. Combining the 2 aspects with sufficient animals illustrates the many problems. Considerable planning is required to undertake research of this type.

The results of this research indicate that experiments of this type can be conducted and should be very worthwhile in providing practical information to improve cattle health and production.

Clinical Signs of Zinc Deficiency in Cattle

Decreased food intake and general depression were the first clinical signs of zinc deficiency. These appeared at a time when the serum zinc values were low (17 µg/dl) and a few days before any skin alterations were detected. The decreased food consumption lasted for only a few days, but the eating pattern of the zinc-deficient calf was changed afterwards to eating a smaller amount more frequently.

A clear progression was observed in the skin changes. These changes started as a rough appearance and loss of brightness of the hair coat

and progressed to areas of alopecia on the legs with finally a cracking and bleeding of the skin, all within approximately 30 days.

Clinical signs similar to the ones found in this experiment were reported in zinc-deficient calves by Miller and Miller (1960, 1962) and by Mills et al. (1967). Contrary to what was reported by the above researchers, ptyalism, stomatitis, overgrowths of the oral mucosa, "bowing" of the hind legs and decreased weight gain were not observed in zinc deficiency in this research. In the same way, contrary to what was reported by Mills et al. (1967), one of the calves in this experiment did not have any clinical signs of zinc deficiency even though its serum zinc concentrations were consistently below 40 µg/dl over a period of 1 month. The highest serum zinc value of this calf during this period was 35 µg/dl.

The differences between the findings in this experiment and the findings of Miller and Miller (1960, 1962) and of Mills et al. (1967) might be due to different amounts of zinc in the rations and to the low food consumption and retarded growth observed in these calves. The rations used by Miller and Miller (1960, 1962) contained 2.7 and 3.6 ppm of zinc, and the one used by Mills et al. (1967) contained from 0.9 to 2 ppm. The basal ration used in this experiment contained approximately 8 ppm of zinc. It is likely that a more acute and severe deficiency was obtained in the experiments by the previous researchers.

The weight gain of 16 kg per calf during the 55 days of the experiment was low. The retarded growth observed in these calves may represent an adjustment to the low zinc ration. Growth has been reported to increase the zinc requirements of cattle (Miller, 1970). The slow growth observed probably resulted in "conserving" zinc, thus lowering

the requirements and diminishing the severity of the clinical signs of zinc deficiency.

Serum Zinc Concentrations

Serum zinc concentrations in the calves ranged from 17 to 83 µg/dl. The highest average was 54 µg/dl for the calves fed only the basal ration. These values are below normal and probably indicate that the zinc requirements of the calves were not being met regardless of the treatment. Values from 80 to 120 µg/dl are given by Mills et al. (1967) as normal plasma zinc concentrations for calves.

Except for the first 2 weeks of the experiment, the serum zinc concentration of the calves did not vary much. During the first 2 weeks occasional 2- to 3-fold differences were observed in samples taken 7 days apart. The small variation observed during most of this experiment suggests that serum zinc concentrations are a reliable means of detecting the zinc status of cattle, provided at least 3 samples are collected at approximately weekly intervals.

Gross Pathology

Thymic hypoplasia was observed in all calves. The heaviest thymic weight was 53 g. Calf thymic weights are given by Venzke (1975) as 100 to 200 g in 1-week-old calves and 400 to 600 g at 4 to 6 weeks of age.

As suggested by the low body weights, the thymic hypoplasia observed in the calves might reflect general malnutrition. Malnutrition is known to cause thymic hypoplasia (Follis, 1958). The thymic hypoplasia might also be due to zinc deficiency. As discussed previously, the serum zinc concentrations were below normal in all the calves. The possible combination of malnutrition and zinc deficiency

in causing the hypoplasia of the thymus observed in this experiment would also have to be considered. The severity of thymic hypoplasia in the calf with the lowest serum zinc concentrations suggests that zinc played an important role in causing this lesion.

Microscopic Pathology

The findings of lymphocytic depletion in the thymuses of the 2 calves with the lowest serum zinc concentrations is in agreement with thymic lymphocytic depletion reported by Brummerstedt et al. (1977) in calves with hereditary zinc deficiency. This also agrees with the findings of Whitenack et al. (1978) in zinc-deficient pigs. It is interesting that both calves had mean serum zinc concentrations below 40 µg/dl. Although the data are too limited to allow any definite conclusion, the possibility that thymic hypoplasia and depletion of lymphocytes in calves occur when the serum zinc values are consistently below 40 µg/dl deserves further consideration.

It is likely that the parakeratosis present in the esophagus, rumen, reticulum and omasum of all calves reflects the lack of roughage in the diet, even though ground corn cobs were used as a source of roughage. Similar findings have been reported by Miller and Miller (1962). The changes described in the lung and abomasum may not be related directly to zinc deficiency.

Effect of $CaNa_2EDTA$ on Serum and Liver Zinc

Calcium disodium EDTA injections effectively lowered serum zinc concentrations, and this effect was dose related since lower values were observed in the calf given 37 µg/kg BW as compared to the calf given half this amount. Contrary to what was expected, zinc supplementation in the amount given was not able to completely prevent the decline of

serum zinc values due to $CaNa_2EDTA$ administration. This finding could be of importance in patients undergoing EDTA therapy for lead poisoning. Mills et al. (1967) demonstrated in calves that when plasma zinc values decreased below 40 µg/dl, clinical signs of zinc deficiency appeared. Such low values are a possibility in patients given large amounts of $CaNa_2EDTA$. Interestingly, lymphocytic depletion in the spleen and lymph nodes of patients given EDTA has been reported (Dudley et al., 1955). This change might be a consequence of altered zinc metabolism in these patients.

Calcium disodium EDTA also lowered the hepatic zinc concentration. As with the serum zinc values, zinc supplementation was not able to completely prevent this decline. It seems, then, that as serum zinc was chelated by $CaNa_2EDTA$ and excreted, hepatic zinc was mobilized. This might indicate that EDTA therapy decreases total zinc body stores.

Toxicity of $CaNa_2EDTA$

The absence of lesions in the kidneys of the calves given $CaNa_2EDTA$ injections was not surprising, considering the findings of Foreman et al. (1956). In their experiments a daily dose of 62.5 mg/kg BW during a 16-day period did not produce any renal changes in rats.

Experimental Enteric Infection

The clinical signs observed in this experiment are in agreement with what has been described for these enteropathogens in younger calves. The incubation period of 7 hours agrees with what has been reported for *E. coli* enteritis.

The absence of gross lesions in all but 1 calf might be due to the recovery of the calves before they were necropsied. The subserosal edema present around the spiral colon of calf 4 is an unusual finding.

The same lesion is sometimes found in pigs with edema disease due to toxigenic *E. coli* (Smith et al., 1972).

The histologic findings and the positive results with fluorescent antibody technique suggested that the coronavirus was the causative agent of the enteritis in calves 9, 11 and 12, even though the short duration of the incubation period is not consistent with this etiology. It is possible, though, that the initial changes were produced by *E. coli* and that these allowed the virus to establish itself and replicate within the epithelial cells. The light *E. coli* growth obtained from intestinal samples is, at least in routine diagnostic work, considered nonsignificant.

No relationship between any of the 4 treatments and susceptibility to the infection was found. The fact that 3 of the calves had severe diarrhea with systemic alterations is in itself unusual since all the calves were more than 7 months old when exposed to *E. coli* and coronavirus. The factors that might have predisposed the calves to the enteric infection could not be identified. Consideration has to be given to both zinc deficiency and malnutrition. The calves were malnourished, as clearly reflected by the small body weight in relation to age. Healthy 6-month-old Holstein calves should weigh from 150 to 200 kg (National Research Council, 1971). The possible effect of the mixed infection as well as the number of microorganisms given also deserves attention. Information on the infective dose of either *E. coli* or coronavirus was not found. The number of bacteria given to the calves in this experiment was not greater than the number given to younger calves by previous researchers (Bellamy and Acres, 1979). No reports of experimentally induced or naturally occurring mixed *E. coli*-coronavirus infections were found. It is possible that the number of viral particles

or bacteria that have been administered to young calves during epxerimental studies is large enough to induce diarrhea even in adult animals. Studies to determine the infective dose for both pathogens would be useful, especially in allowing a better comparison of results obtained by different researchers.

The presence of *E. coli* enterotoxin in the culture media used as inoculum cannot be ruled out, and the possibility of this enterotoxin being the promoter of diarrhea in the 3 mentioned calves cannot be excluded. Lack of previous exposure to *E. coli* and coronavirus also might have played a role in determining susceptibility, since the calves were separated from other cattle when still less than 1 week old. Nevertheless, the isolation of hemolytic *E. coli* from 1 diarrheic calf that was in the initial group of 12 tends to invalidate this consideration for *E. coli*.

Influence of *E. coli* and Coronavirus Diarrhea on the Serum Zinc, Total Protein and Albumin

Lowered serum zinc values were observed after the infection in the 3 calves with severe diarrhea. Two of these calves also had lowered serum total protein and albumin concentrations. Serum zinc concentrations decreased to about half the previous values, 24 hours after inoculation. This decrease occurred in all clinically sick calves independently of their zinc status. This finding differs from what was reported by Whitenack et al. (1978). In their study with TGE-infected piglets, lower serum zinc values were not observed in zinc-deficient piglets after infection.

Decreased serum zinc and albumin values during diseased states have been attributed to the action of leukocytic endogenous mediator (LEM) (Powanda, 1979). In several reports on the action of LEM, no

reference is made to changes in serum total protein. Lowered serum zinc, total protein and albumin concentrations found in this study might be due to the action of this mediator. On the other hand, the concomitance of changes in albumin and total protein might just indicate an increased protein catabolism resulting from increased basal metabolic rate due to fever. Negative nitrogen balance has been found during febrile diseases (Beisel, 1972). These changes might also reflect the exudation of proteins into the intestine.

SUMMARY

Six, 6-month-old calves were used to determine the clinical signs and pathology of zinc deficiency and their susceptibility to an enteric infection. All 6 calves were fed a basal, low zinc ration for 50 days. Four of the 6 calves were injected with different amounts of $CaNa_2EDTA$ and 2 of these 4 were orally supplemented with zinc. At the end of 50 days of these treatments, all calves were exposed to *E. coli* and coronavirus and necropsied 5 days later.

The clinical signs associated with zinc deficiency were inappetence, apathy and skin changes. These changes consisted of alopecia, scaliness and bleeding fissures of the skin, especially on the legs. Serum and hepatic zinc values were lowest in calves injected with $CaNa_2EDTA$ and not supplemented with zinc. Microscopically, there was a depletion of lymphocytes in the thymuses of calves with low serum and hepatic zinc values. Exposure to *E. coli*-coronavirus produced a diarrhea in 3 of the 6 calves. In 2 of these 3 calves with diarrhea, serum zinc, albumin and total protein values were decreased. Histologically, the intestinal villi of calves with diarrhea were denuded of epithelium or covered by a cuboidal type of epithelial cells.

Zinc-deficient calves were susceptible to enteric infection and the resulting diarrhea decreased serum and hepatic zinc concentrations.

BIBLIOGRAPHY

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Ahrens, F. A., and Aronson, A. L.: A comparative study of the toxic effects of calcium and chromium chelates of ethyelediamine-tetraacetate in the dog. Toxicol. Appl. Pharmacol., 18, (1971): 10-25.

Altman, J., Wakim, K. G., and Winkelmann, R. K.: Effects of edathamil-disodium on the kidney. J. Invest. Dermatol., 38, (1962): 215-218.

Andresen, E., Flagstad, T., Basse, A., and Brummerstedt, E.: Evidence of a lethal trait, A46, in black pied Danish cattle of Friesian descent. Nord. Vet.-Med., 22, (1970): 473-485.

Beisel, W. R.: Interrelated changes in host metabolism during generalized infectious illness. Am. J. Clin. Nutr., 25, (1972): 1254-1260.

Bellamy, J. E. C., and Acres, S. D.: Enterotoxigenic colibacillosis in colostrum-fed calves: pathologic changes. Am. J. Vet. Res., 40, (1979): 1391-1397.

Blood, D. C., Henderson, J. A., and Radostits, O. M.: *Veterinary Medicine,* 5th Ed. Lea and Febiger, Philadelphia, (1979).

Brummerstedt, E., Andresen, E., Basse, A., and Flagstad, T.: Lethal trait A46 in cattle. Immunological investigations. Nord. Vet.-Med., 26, (1974): 279-293.

Brummerstedt, E., Basse, A., Flagstad, T., and Andresen, E.: Animal model of human disease. Acrodermatitis enteropathica, zinc malabsorption. Am. J. Path., 87, (1977): 725-728.

Brummerstedt, E., Flagstad, T., Basse, A., and Andresen, E.: The effect of zinc on calves with hereditary thymus hypoplasia (lethal trait A46). Acta Path. Microbiol. Scand., 79 (section A), (1971): 686-687.

Demertzis, P. N., and Mills, C. F.: Oral zinc therapy in the control of infectious pododermatitis in young bulls. Vet. Rec., 93, (1973): 219-222.

Doolan, P. D., Schwartz, S. L., Hayes, J. R., Mullen, J. C., and Cummings, N. B.: An evaluation of the nephrotoxicity of ethylenediamine-tetraacetate and diethylenetriaminepentaacetate in the rat. Toxicol. Appl. Pharmacol., 10, (1967): 481-500.

Dubowsky, K. M.: Zinc. Type C procedure. In: *CRC Manual of Analytical Toxicology*, ed. I. Sunshine. The Chemical Rubber Company, Cleveland, OH, (1971): 344-347.

Dudley, H. R., Ritchie, A. C., Schilling, A., and Baker, W. H.: Pathologic changes associated with the use of sodium ethylene diamine tetra-acetate in the treatment of hypercalcemia. New Engl. J. Med., 252, (1955): 331-337.

Dynna, O., and Havre, G. N.: Interrelationship of zinc and copper in the nutrition of cattle. A complex zinc-copper deficiency. Acta Vet. Scand., 4, (1963): 197-208.

Edwards, H. M., Jr., Young, R. J., and Gillis, M. B.: Studies on zinc in poultry nutrition. 1. The effect of feed, water and environment on zinc deficiency in chicks. Poultry Sci., 37, (1958): 1094-1099.

England, J. J.: Viruses associated with neonatal diarrhea of calves. Vet. Med./S.A.C., 72, (1977): 925-927.

Flagstad, T.: Lethal trait A46 in cattle. Intestinal zinc absorption. Nord. Vet.-Med., 28, (1976): 160-169.

Follis, R. H., Jr.: *Deficiency Disease*. Charles C. Thomas, Springfield, IL, (1958).

Foreman, H., Finnegan, C., and Lushbaugh, C. C.: Nephrotoxic hazard from uncontrolled edathamil calcium-disodium therapy. J. Am. Med. Assoc., 160, (1956): 1042-1046.

Fraker, P. J., Haas, S. M., and Luecke, R. W.: Effect of zinc deficiency on the immune response of the young adult A/J mouse. J. Nutr., 107, (1977): 1889-1895.

Frankel, S., Reitman, S., and Sonnenwirth, A. C. (ed.): *Gradwohl's Clinical Laboratory Methods and Diagnosis*, Vol. 2, 7th Ed. The C. V. Mosby Co., St. Louis, MO, (1970).

Golden, M. H. N., Jackson, A. A., and Golden, B. E.: Effect of zinc on thymus of recently malnourished children. Lancet, (1977): 1057-1059.

Golias, T.: *Helena Electrophoresis Manual*. Helena Laboratories, Beaumont, TX, (1976).

Greaves, M., and Boyde, T. R. C.: Plasma zinc concentrations in patients with psoriasis, other dermatoses and venous leg ulceration. Lancet, (1967): 1019-1020.

Haaranen, S.: The effect of zinc on itching tail root eczema in cattle. Nord. Vet.-Med., 14, (1962): 265-269.

Haaranen, S.: Some observations on the zinc requirement of cattle for the prevention of itch and hair licking at different calcium levels in the feed. Nord. Vet.-Med., 15, (1963): 536-542.

Haaranen, S.: Some observations on the occurrence of itch and hair licking in cattle at different zinc and copper levels in the feed. Nord. Vet.-Med., 17, (1965): 36-38.

Halsted, J. A., and Smith, J. C., Jr.: Plasma-zinc in health and disease. Lancet, (1970): 322-324.

Hambidge, K. M., Hambidge, C., Jacobs, M., and Baum, J. D.: Low levels of zinc in hair, anorexia, poor growth, and hypogeusia in children. Pediat. Res., 6, (1972): 868-874.

Hove, E., Elvehjem, C. A., and Hart, E. B.: The physiology of zinc in the nutrition of the rat. Am. J. Physiol., 119, (1937): 768-775.

Kernkamp, H. C. H., and Ferrin, E. F.: Parakeratosis in swine. J. Am. Vet. Med. Assoc., 123, (1953): 217-220.

Langpap, T. J., Bergeland, M. E., and Reed, D. E.: Coronaviral enteritis of young calves: virologic and pathologic findings in naturally occurring infections. Am. J. Vet. Res., 40, (1979): 1476-1478.

Legg, S. P., and Sears, L.: Zinc sulphate treatment of parakeratosis in cattle. Nature, 186, (1960): 1061-1062.

Levine, W. G.: Heavy metals and heavy-metal antagonists. In: *The Pharmacological Basis of Therapeutics,* 5th Ed., ed. L. S. Goodman and A. Gilman. Macmillan Publishing Co., Inc., New York, (1975): 912-923.

Lewis, L. D., and Phillips, R. W.: Pathophysiologic changes due to coronavirus-induced diarrhea in the calf. J. Am. Vet. Med. Assoc., 173, (1978): 636-642.

Lewis, P. K., Jr., Hoekstra, W. G., and Grummer, R. H.: Restricted calcium feeding versus zinc supplementation for the control of parakeratosis in swine. J. Anim. Sci., 16, (1957): 578-588.

Luecke, R. W., Olman, M., and Baltzer, B.: Zinc deficiency in the rat: effect on serum and intestinal alkaline phosphatase activities. J. Nutr., 94, (1968): 344-350.

Luna, L. G. (ed.): *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology,* 3rd Ed. McGraw-Hill Book Co., New York, (1968).

Mebus, C. A., Newman, L. E., and Stair, E. L., Jr.: Scanning electron, light, and immunofluorescent microscopy of intestine of gnotobiotic calf infected with calf diarrheal coronavirus. Am. J. Vet. Res., 36, (1975): 1719-1725.

Mebus, C. A., Stair, E. L., Rhodes, M. B., and Twiehaus, M. J.: Pathology of neonatal calf diarrhea induced by a coronavirus-like agent. Vet. Path., 10, (1973): 45-64.

Miller, E. R., Luecke, R. W., Ullrey, D. E., Baltzer, B. V., Bradley, B. L., and Hoefer, J. A.: Biochemical, skeletal and allomeric changes due to zinc deficiency in the baby pig. J. Nutr., 95, (1968): 278-286.

Miller, J. K., and Miller, W. J.: Development of zinc deficiency in Holstein calves fed a purified diet. J. Dairy Sci., 43, (1960): 1854-1856.

Miller, J. K., and Miller, W. J.: Experimental zinc deficiency and recovery of calves. J. Nutr., 76, (1962): 467-474.

Miller, W. J.: Zinc nutrition of cattle: a review. J. Dairy Sci., 53, (1970): 1123-1135.

Miller, W. J., Blackmon, D. M., Gentry, R. P., Powell, G. W., and Perkins, H. F.: Influence of zinc deficiency on zinc and dry matter content of ruminant tissues and on excretion of zinc. J. Dairy Sci., 49, (1966): 1446-1453.

Miller, W. J., Clifton, C. M.,and Cameron, N. W.: Zinc requirement of Holstein bull calves to nine months of age. J. Dairy Sci., 46, (1963): 715-719.

Miller, W. J., Clifton, C. M., Fowler, P. R., and Perkins, H. F.: Influence of high levels of dietary zinc on zinc in milk, performance and biochemistry of lactating cows. J. Dairy Sci., 48, (1965b): 450-453.

Miller, W. J., Pitts, W. J., Clifton, C. M., and Morton, J. D.: Effects of zinc deficiency per se on feed efficiency, serum alkaline phosphatase, zinc in skin, behavior, greying and other measurements in the Holstein calf. J. Dairy Sci., 48, (1965a): 1329-1334.

Miller, W. J., Pitts, W. J., Clifton, C. M., and Schmittle, S. C.: Experimentally produced zinc deficiency in the goat. J. Dairy Sci., 47, (1964): 556-559.

Mills, C. F., Dalgarno, A. C., Williams, R. B., and Quarterman, J.: Zinc deficiency and the zinc requirements of calves and lambs. Br. J. Nutr., 21, (1967): 751-768.

Morin, M., Lariviere, S., Lallier, R., Begin, M., Roy, R., and Ethier, R.: Neonatal calf diarrhea: pathology and microbiology of spontaneous cases in dairy herds and incidence of the enteropathogens implicated as etiological agents. Proc. Second International Symposium on Neonatal Diarrhea, (1978): 347-367.

National Research Council: Nutrient requirements of dairy cattle. National Academy of Sciences, Washington, DC, (1971).

National Research Council: Nutrient requirements of beef cattle. National Academy of Sciences, Washington, DC, (1976).

National Research Council: Nutrient requirements of dairy cattle. National Academy of Sciences, Washington, DC, (1978).

Neathery, M. W., Miller, W. J., Blackmon, D. M., and Gentry, R. P.: Performance and milk zinc from low-zinc intake in Holstein cows. J. Dairy Sci., 56, (1973): 212-217.

Neumann, C. G., Lawlor, G. J., Jr., Stiehm, E. R., Swendseid, M. E., Newton, C. Herbert, J., Ammann, A. J., and Jacob, M.: Immunologic responses in malnourished children. Am. J. Clin. Nutr., 28, (1975): 89-104.

Newman, L. E., Whitehair, C. K., and Mullaney, T. P.: Immune response of the bovine fetus to *in utero* vaccination with bovine coronavirus. Proc. Second International Symposium on Neonatal Diarrhea, (1978): 457-462.

O'Dell, B. L., and Savage, J. E.: Symptoms of zinc deficiency in the chick. Fed. Proc., 16, (1957): 394.

Ott, E. A., Smith, W. H., Stob, M., and Beeson, W. M.: Zinc deficiency syndrome in the young lamb. J. Nutr., 82, (1964): 41-49.

Ott, E. A., Smith, W. H., Stob, M., Parker, H. E., and Beeson, W. M.: Zinc deficiency syndrome in the young calf. J. Anim. Sci., 24, (1965): 735-741.

Pearson, G. R., McNulty, M. S., and Logan, E. F.: Pathological changes in the small intestine of neonatal calves with enteric colibacillosis. Vet. Path., 15, (1978): 92-101.

Pekarek, R. S., and Beisel, W. R.: Characterization of the endogenous mediator(s) of serum zinc and iron depression during infection and other stresses. Proc. Soc. Exp. Biol. Med., 138, (1971): 728-732.

Pekarek, R. S., Hoagland, A. M., and Powanda, M. C.: Humoral and cellular immune responses in zinc deficient rats. Nutr. Rep. Inter., 16, (1977): 267-276.

Pekarek, R. S., Wannemacher, R. W., Jr., and Beisel, W. R.: The effect of leukocytic endogenous mediator (LEM) on the tissue distribution of zinc and iron. Proc. Soc. Exp. Biol. Med., 140, (1972): 685-688.

Perry, H. M., and Perry, E. F.: Normal concentrations of some trace metals in human urine: changes produced by ethylenediaminetetraacetate. J. Clin. Invest., 38, (1959): 1452-1463.

Perry, T. W., Beeson, W. M., Smith, W. H., and Mohler, M. T.: Value of zinc supplementation of natural rations for fattening beef cattle. J. Anim. Sci., 27, (1968): 1674-1677.

Powanda, M. C.: Host metabolic alterations during inflammatory stress in reference to nutritional status. Proc. 116th Annual American Veterinary Medical Association Meeting, (1979): 194.

Prasad, A. S.: Trace elements: biochemical and clinical effects of zinc and copper. Am. J. Hematol., 6, (1979a): 77-87.

Prasad, A. S.: Clinical, biochemical, and pharmacological role of zinc. Ann. Rev. Pharmacol. Toxicol., 20, (1979b): 393-426.

Quarterman, J.: The effects of zinc deficiency or excess on the adrenals and the thymus in the rat. In: *Trace Element Metabolism in Animals*, Vol. 2, ed. W. G. Hoekstra, J. W. Suttie, H. E. Ganther, and W. Mentz. University Park Press, Baltimore, MD, (1974): 742-748.

Reed, D. E., Bicknell, E. J., Larson, C. A., Knudtson, W. U., and Kirkbride, C. A.: Infectious bovine rhinotracheitis virus-induced abortion: rapid diagnosis by fluorescent antibody technique. Am. J. Vet. Res., 32, (1971): 1423-1426.

Robertson, B. T., and Burns, M.J.: Zinc metabolism and the zinc-deficiency syndrome in the dog. Am. J. Vet. Res., 24, (1963): 997-1002.

Sharpee, R. L., Mebus, C. A., and Bass, E. P.: Characterization of a calf diarrheal coronavirus. Am. J. Vet. Res., 37, (1976): 1031-1041.

Smith, H. A., Jones, T. C., and Hunt, R. D.: *Veterinary Pathology*, 4th Ed. Lea and Febiger, Philadelphia, (1972).

Stake, P. E., Miller, W. J., and Gentry, R. P.: Zinc metabolism and homeostasis in ruminants as affected by dietary energy intake and growth rate. Proc. Soc. Exp. Biol. Med., 142, (1973): 494-496.

Todd, W. R., Elvehjem, C. A., and Hart, E. B.: Zinc in the nutrition of the rat. Am. J. Physiol., 107, (1934): 146-156.

Tucker, H. F., and Salmon, W. D.: Parakeratosis or zinc deficiency in the pig. Proc. Soc. Exp. Biol. Med., 88, (1955): 613-616.

Tyrrell, D. A. J., Alexander, D. J., Almeida, J. D., Cunningham, C.H., Easterday, B. C., Garwes, D. J., Hierholzer, J. C., Kapikian, A., Macnaughton, M. R., and McIntosh, K.: Coronaviridae: second report. Intervirology, 10, (1978): 321-328.

Underwood, E. J., and Somers, M.: Studies of zinc nutrition in sheep. 1. The relation of zinc to growth, testicular development and spermatogenesis in young rams. Aust. J. Agr. Res., 20, (1969): 889-897.

Venzke, W. G.: Thymus. In: *Sisson and Grossman's The Anatomy of the Domestic Animals*, 5th Ed., ed. R. Getty. W. B. Saunders Co., Philadelphia, (1975): 1064.

Voelker, H. H., Jorgensen, N. A., Mohanty, G. P., and Owens, M. J.: Effect of zinc supplementation to dairy cattle rations. J. Dairy Sci., 52, (1969): 929-930.

Weismann, K., and Flagstad, T.: Hereditary zinc deficiency (adema disease) in cattle, an animal parallel to acrodermatitis enteropathica. Acta Dermatovener. (Stockholm), 56, (1976): 151-154.

Whitenack, D. L., Whitehair, C. K., and Miller, E. R.: Influence of enteric infection on zinc utilization and clinical signs and lesions of zinc deficiency in young swine. Am. J. Vet. Res., 39, (1978): 1447-1454.

Young, R. J., Edwards, H. M., Jr., and Gillis, M. B.: Studies on zinc in poultry nutrition. 2. Zinc requirement and deficiency symptoms of chicks. Poultry Sci., 37, (1958): 1100-1107.

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