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THE PATHOLOGY OF THE SKIN IN ZINC DEFICIENT CALVES, CHICKS AND SWINE

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THE PATHOLOGY OF THE SKIN IN ZINC DEFICIENT CALVES, CHICKS AND SWINE

By

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ABSTRACT

THE PATHOLOGY OF THE SKIN IN ZINC DEFICIENT CALVES, CHICKS AND SWINE

By

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Five experiments were conducted using 20 calves, 148 chicks and 12 pigs to determine the morphologic and pathologic features of the integumentary tissue, especially the skin, during health and zinc deficiency. In addition, the comparative dermatopathology of zinc deficiency in these 3 species was evaluated. The interrelationships between zinc deficiency and an experimental epidermal infection were also determined in calves exposed to *Dermatophilus congolensis* and pigs exposed to *Staphylococcus hycus*. Zinc deficiency was characterized by serum zinc values, hematological determinations, protein electrophoresis, clinical signs, and gross and microscopic lesions. The same techniques were used in all 3 species.

In calves the clinical signs of zinc deficiency were inappetence, hypersalivation, tender fetlocks, alopecia and crusted skin lesions involving especially the legs. In chicks the clinical signs of zinc deficiency were inappetence, reduced growth rate, poor and abnormal feathering, abnormal gait and inability to stand, scaly dry skin and thickening of foot pads. In pigs the clinical signs were inappetence, reduced growth rate and crusted, erythematous epidermal lesions on the face, ears, and feet. Serum zinc values were decreased in all 3

species and the values correlated with the manifestations of zinc deficiency. A dermal biopsy instrument was most useful in following the microscopic skin changes during the deficiency in calves and pigs. In calves the microscopic lesions consisted of a parakeratotic stratum corneum, acanthosis and elongated rete pegs that anastomosed with each other. In severe cases the stratum granulosum was replaced by spongiosis except in areas around the hair follicles. In chicks, microscopic lesions included hyperkeratosis and acanthosis with dyskeratosis in the foot pads. The epidermis of the feather follicles on the wings was parakeratotic and acanthotic. In pigs the epidermal lesions were parakeratosis and acanthosis with elongation and anastomosis of rete pegs. In all 3 species the epidermal lesions were similar and involved primarily the keratinocyte. In the chick, however, the hyperkeratosis was more prominent than in the calf and pig. Parakeratosis was present in areas of the wing. Abrasions and presence of microorganisms were factors that modified the nature of the lesions.

Skin lesions of *D. congolensis* infection were more severe in zinc-deficient than in zinc-supplemented calves and consisted of a parakeratotic stratum corneum, acanthosis, spongiform abscesses and hidradenitis. Typical clinical signs of *S. hycus* infection were not produced in pigs, but in the zinc-deficient pigs the hair follicles had a suppurative folliculitis related to gram-positive microorganisms.

Zinc deficiency has a similar effect on the integumentary tissue of calves, chicks and pigs. The tissue changes would increase susceptibility to microbial, parasitic, or chemical injury. Supplemental zinc in livestock rations would most likely improve resistance to skin diseases.

DEDICATION

to my wife and to my son

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INTRODUCTION

Skin diseases of livestock are a very important problem in countries like Brazil. The economic losses associated with the value of the hides have been increasing annually. The control and prevention of ectoparasites such as the human bot-fly (*Dermatobia hominis* Linaeus Jr.), the screw-worm (*Cochliomyia hominivorax* Coquerel), ticks (mainly *Boophilus microplus* [Canestrini]), mange, and tick-borne diseases (babiosis and anaplasmosis) would greatly improve livestock production. Other diseases, such as streptotrichosis (*Dermatophilus congolensis* Van Saceghan) and ringworm (*Trichophyton* spp.), have been associated with parasites, but little is known of their influence. Injuries to the skin that might subsequently be infected by parasites or pathogens are also an important problem.

Examination of the skin involves a study of the gross and microscopic components. Many researchers consider the skin to be an organ which lends itself well to such an examination. The skin is subjected to a variety of pathological processes which stem from its exposure to influences mediated by the environment, causing physical or chemical injury. The main function of the skin is to act as a barrier, preventing the loss of the body constituents and protecting against environmental changes. When pathological skin changes of any type occur, they cause a breakdown of the barrier function and secondary cutaneous, subcutaneous or systemic reactions.

Nutritional deficiencies, due to their negative effect on the integrity of tissues such as the skin, have long been associated with a decreased resistance to infection.

Zinc has been considered for many years as one of the important trace elements, and a deficiency leads to skin changes and systemic disorders. The pathological changes of the skin during zinc deficiency have not been clearly described in some species. A severe parakeratosis has been considered to be an important clinical sign and lesion in swine. In other animals and man, less emphasis has been given to the role of zinc in the pathology of the integumentary system. However, the clinical, nutritional and metabolic aspects of zinc deficiency have received considerable research attention.

This research would supply important additional information on the effect of zinc deficiency on the integumentary system of calves, chicks and pigs and on the role of zinc in resistance to infection.

REVIEW OF LITERATURE

This literature review is on morphology of the normal skin, the pathological changes in the skin of zinc deficiency in man and animals, and the role of zinc in resistance to infection.

Zinc has been reported to be an essential nutrient for normal growth and development in man and animals. It was first used as an ointment for skin diseases by ancient civilizations (NRC, 1979) and was documented experimentally to be of value by Graham (1826). Todd et al. (1934) reported that zinc was an essential nutrient for the normal development of the rat. A severe parakeratosis in swine was described by Kernkamp and Ferrin (1953), and this was reported later to be related to a zinc deficiency (Tucker and Salmon, 1958). In chickens, retarded growth, lack of feathering and severe dermatitis of the feet were attributed to zinc defieincy (Roberson and Schaible, 1958). In 1960, Legg and Sears reported that parakeratotic lesions in grazing cattle responded to zinc therapy. Subsequently, the work of Haaranen and Hyppolà (1961) in Finland indicated that a condition known as "hair slicking itch" in dairy cows also responded to oral zinc therapy. Miller and Miller (1960) described an acute experimental zinc deficiency in dairy calves. More recently, Dermetzis and Mills (1973) associated the susceptibility of young bulls to infectious pododermatitis with low serum levels of zinc. Skin lesions are a manifestation of zinc deficiency in most species, including man.

Morphology and Pathophysiology of the Skin

The most important function of the skin is to provide an anatomic and physiologic barrier between the animal and its environment. The skin is composed of two layers. The first is the outer layer, or epidermis, which is responsible for protecting the body against external injuries. The second is the dermis, or corion, that is responsible for nourishment of the epidermis. The appendages consist of sebaceous, sweat glands and hair or feathers, which are also responsible for protection and for regulation of the body temperature.

The skin, like any organ, is subjected to a variety of pathologic conditions. Each functional disorder is accompanied by morphological, nutritional and endocrine changes.

Epidermis

Anatomically, the epidermis is divided into the following zones: the basal layer, the prickle layer, the granular layer and the keratin layer. An additional layer, the stratum lucidum, is described, but it is only clearly evident in the epidermis of the foot pads. These divisions were considered by Jarret (1973) as being indistinct anatomical entities which tended to gradually merge with each other.

Dermis

The dermis is basically composed of distinct structures, especially fibers, ground substance and cells. The fibers are mostly collagen. The ground substance is composed of hyaluronic acid and chondroitin sulfuric acid. There are three types of dermal cells: (1) fibroblasts, which are responsible for producing the collagen fibers; (2) mastocytes, which occur in all areas of connective tissue, especially around blood vessels and which are responsible for releasing chemical factors

like histamine and serotonin; and (3) histiocytes, which are responsible for phagocytosis. These cells have an important role in inflammatory processes (Miller and Kirk, 1976).

Morphological and Physiological Changes

Pathologic processes may primarily involve the epidermis, the dermis, or both areas. Basically, the changes are congenital or acquired and localized or diffuse. The keratinocytes are abundant cells whose major function is to elaborate the horny layer of the epidermis.

According to Bullough (1972), the process of keratinocyte maturation and intracellular keratin formation depends upon a dynamic equilibrium between basal layer division, migration, maturation and shedding. If production exceeds shedding, the epidermis increases in size and is called acanthosis. If the stratum corneum increases in keratinization, it is called hyperkeratosis. If the corneal layer does not retain its nuclei, the condition is characterized as hyperkeratosis. If the corneal cells retain their nuclei, the condition is called parakeratosis.

Varying degrees of injury produce changes ranging from erythema, due to secondary dermal vasodilatation, and bullous formation. Spongiosis is characterized by the appearance of vesicular areas of free inter- and intracellular fluid (Baker, 1975).

In other circumstances the keratinocyte injury is localized in specific areas. Viruses may involve the cellular cytoplasm (pox virus) or the nucleus (wart virus). Desmosomal complexes may become abnormal, leading to reduced intercellular adhesion and giving the appearance of acantholysis of pemphigus (Braum-Falco, 1969). The

maturation of the epidermal cells can be disorganized, while individual cell keratinization may progress to intraepidermal carcinoma or an ectodermal defect such as ichthyosis (Miller and Kirk, 1976).

Disorders of pigmentation are associated with altered melanin production by the melanocyte. The melanin is injected via the dendritic processes into surrounding keratinocytes. Melanin production may be excessive, due to endocrine disturbances, chronic inflammatory processes or irritation, and lead to hyperpigmentation. The lack of tyrosinase for melanin synthesis may lead to hypopigmentation (albinism). Finally, proliferations of abnormal junctional melanocytes later in life are considered the first feature of malignant melanomas (Baker, 1975).

A decrease of collagen fibers is associated with a hereditary disease known as cutaneous asthenia in dogs. In this disease, fibrous dysplasia of connective tissue occurs (Hegreberg and Padgett, 1967). Other disorders, like inflammatory reactions, neoplasms, and blood vessel dyscrasias, are important changes occurring in the corion.

Endocrine Disturbances

Endocrine substances have a potent effect on the skin. In the normal skin, the hormonal function is characterized by a precise balance between the synthesis and degradation of cells. The most frequent endocrine disturbances are: hyperestrogenism, hypothyroidism and hyperadrenalism.

Hyperestrogenism can result from the prolonged administration of estrogens, the presence of a Sertoli cell tumor of the testicle, or disturbances of the ovary. Hair loss occurs on bilaterally

symmetrical areas of the legs and occasionally on the entire body. The hair becomes brittle with depletion of the hair follicles and sebaceous glands and subsequent atrophy of the skin (Amoroso and Ebling, 1966).

In hypothyroidism, the skin becomes atrophic, smooth and hyperkeratotic. The hair which remains over the body is fine and sparse. Edema of the corion is common. In general, the lesions resemble hyperestrogenism (Thomsett, 1966).

In hyperadrenocorticism, there is a bilateral alopecia of the ventral and lateral aspects of the body. The skin is atrophic due to the effect of increased cortisol levels on the mitotic activity of the cell. There is prominent hyperkeratosis and a reduction in numbers of the hair follicles, apocrine and sebaceous glands. The hair follicles are filled with keratin. Focal mineralization along collagen and elastic fibers is common in this disorder (Ettinger, 1975).

Nutritional Disturbances

The skin has been evaluated in many experimental nutritional deficiencies. In general, the skin provides an excellent guide to the state of nutrition and health. Experimental information on the role of nutrition in the development of skin lesions is well summarized by Follis (1958).

In general, the skin appears to be no different from other tissues in its basic metabolic processes. Atrophy of the epidermis and its appendages is present in malnutrition as a result of calorie, protein and riboflavin deficiencies. Hyperkeratosis followed by acanthosis and, less frequently, parakeratosis are observed in zinc, pantothenic acid, pyridoxine, biotin, vitamin A and essential fatty acid deficiencies.

Specific disturbances related to pigment production are restricted to hair color. Graying, or achromotrichia, is noted in copper, cystine, pantothenic acid and para-aminobenzoic acid deficiencies. Alterations, leading to alopecia in animals, are associated with the integrity of the follicular cells. The cyclic activity of follicular cells is affected by zinc, biotin, inositol, and riboflavin deficiencies. Changes in the sebaceous glands consisting of hypertrophy of the cells are described in zinc-deficient animals, while necrosis is present in riboflavin-deficient rats. The failure to grow collagen fibers in the dermis is a direct result of riboflavin deficiency, while ascorbic acid deficiency is responsible for interference in the formation of collagen.

Physiological responses of the blood vessels, such as dilatation, are associated with magnesium and pyridoxine deficiencies. Vascular defects are associated with ascorbic acid deficiency.

Zinc and the Integumentary System

Zinc has been recognized as an important trace element responsible for several physiological functions in maintaining the integrity of the skin. Zinc functions mainly as a cofactor for important enzymes, including carbonic anhydrase, carboxypeptidase, uricase, phosphatases and several dehydrogenases.

Im et al. (1975) reported that the activity of enzymes involved in glycolysis of the epidermis was decreased by 30 to 50% in zinc-deficient rats in comparison to rats fed normal amounts of zinc. The most important decreases were in phosphofructokinase, glyceraldehyde-3-phosphatase, glucose-6-phosphate, glutamate dehydrogenases, fumarate hydratase and aminotransferases. These decreases suggested that zinc deficiency had also caused a general depression of protein synthesis.

In an electron microscopic analysis of the dermis, Hsu et al. (1974) observed that fibroblasts in zinc-deprived rats were smaller and had an increase in ratio between nucleus and cytoplasm. There were few organelles and the rough endoplasmic reticulum, which was decreased, indicated a possible failure in ribosomal formation. The Golgi apparatus was also smaller. In general, the cell presented irregular contours with a small number of pinocytotic vesicles. The nuclei also had irregular contours. Finally, the fibroblasts of zinc-deficient rats had the signs of cellular immaturity. Voelker (1970), using a light microscope, reported that fibroblasts appeared active and immature around the perivascular regions of the dermis of zinc-deficient pigs.

The skin of the zinc-deficient rat appeared grossly thinner, according to Hsu et al. (1974). The quantity of connective tissue was difficult to evaluate morphologically. The diameter of collagen fibers was also variable in the cellular portion of dense collagen bundles. The cross linking of some fibers had partially disappeared and they were gradually replaced by amorphous components and spherular material. In contrast, the collagen fibers of zinc-supplemented rats were smooth, regular in size and uniformly arranged with distinct striations.

Reaven and Cox (1962) stained sections of normal human skin for zinc, using the dithizone method. A homogeneous, moderately positive stain was obtained for the epidermis, arrectores pilorum, sweat glands and ducts, outer root shafts of hair follicles and the muscular layers of large blood vessels. The connective tissue of the dermis remained unstained. An intense zinc-positive stain was observed in the granules of the stratum granulosum. This was not observed in the skin of

psoriatic patients, suggesting that zinc-dependent enzymes participate in the keratinization of the normal epidermis.

Klevay (1970), using hair from human subjects, discovered a distinct correlation between the zinc content of hair and age. The zinc values decreased gradually during the first period of life and increased during the second. No correlation due to sex was demonstrated between the zinc content of the hair and plasma, between hair and red blood cells, and between plasma and red blood cells. Similar results between zinc hair values and age were reported in zinc-deficient calves (Miller et al., 1965).

Reinhold et al. (1968) reported that zinc concentration was decreased in hair of weanling rats fed diets low in zinc. However, this effect was demonstrated only during the period of zinc depletion.

The morphological characteristics of hair roots from patients with classical marasmus consisted of a striking shift to the resting phase of hair growth (Bradfield et al., 1969). In other research Bradfield (1971) observed that patients with protein-calorie malnutrition had diminished, atrophic, depigmented bulbs and an absence of the sheath. When protein was added to the diet, the hair quality improved. These changes were not observed in experimentally zinc-deficient pigs, except that the majority of the hair shafts in the dermatitic areas were found in the resting phase (Voelker, 1970).

Voelker (1970) reported that the apocrine sweat glands in zinc-deficient pigs were hypertrophic. The glands were even more hypertrophic in infected skin. In addition, the sebaceous glands did not have remarkable changes, because in young pigs these glands had not completely developed at this age. In zinc-deficient rats, they had become hypertrophic (Follis et al., 1941).

Zinc and Skin Diseases

According to Sunderman (1975), in man acrodermatitis enteropathica has been considered to be an autosomal recessive disease characterized by low zinc serum values and progressive bullous-pustular dermatitis. The lesions involved the external mucosal surfaces. The nails had a generalized alopecia. Microscopically, the lesions were consistent with hyperkeratosis, parakeratosis, and acanthosis of the stratum spinosum with severe and localized spongiosis and elongation of the rete pegs. In extreme cases, severe acantholysis was observed in the epidermis (Juljulian and Kurban, 1971).

In cattle, a condition known as "adamae" disease was found in black-footed Danish castle. The calves appeared normal at birth, but skin lesions began at the age of 4 to 8 weeks. The lesions were characterized by exanthema, alopecia and severe parakeratosis around the mouth surface and under the jaw (Andresen et al., 1974). Later the lesions often became severely infected by bacteria or fungi (Andresen et al., 1974; Sunderman, 1975).

In man, psoriasis, a localized skin disorder characterized by loss of a large number of skin cells, may be associated with depletion of zinc in the body (Prasad, 1979). Lesions of psoriasis were considered by Greaves and Boyde (1967) to be the same as those reported in zinc-deficient animals.

An imbalance of zinc and other minerals was reported to be responsible for the "rat tail" syndrome in cattle (Irwil, 1979) and itching tail root eczema in dairy cattle (Haaranen, 1962).

Greaves and Boyde (1967) mentioned that plasma zinc concentration was lowered in patients with venous leg ulceration and in other

dermatological diseases, such as ichthyosis, lichen planus, eczema, urticaria, scleroderma, pemphigoid, dermatitis herpetiformis, rosacea and chronic discoid lupus erythematosus.

Zinc Metabolism in Systemic Infections

Zinc metabolic alterations have been recognized during infection. Serum zinc concentration is known to decline suddenly during an infectious process. In 1951, Vikbladh was the first to have observed changes in serum zinc values in Scandinavian patients, and subsequently these findings were reported in experimental animals and man with bacterial (Powanda et al., 1975), rickettsial (Beisel and Pekarek, 1972) and viral (Pekarek et al., 1970; Squibb et al., 1972) infections.

Depletion of zinc in the serum was also observed following the administration of endotoxins from *Escherichia coli* or *Diplococcus pneumoniae* and from administration of attenuated vaccine of *Francisella tularensis* to rats by Pekarek and Beisel (1971).

Storage forms of zinc in the body have not been found. However, in normal persons 32% of the zinc is tightly bound to $\alpha_2$macroglobulin and 66% to serum albumin, and 2% occurs as a microligant with some amino acids (Henkin, 1974).

Beisel (1976) pointed out that in the early stage of infectious process a significant decrease of serum zinc concentration occurs. Concomitantly, there is an increase in uptake of zinc by the liver. Eddington et al. (1971) concluded that zinc may be transported to the liver to meet the increased requirements for protein synthesis. However, when the infection becomes subacute or chronic the body zinc balance may shift to negative values due to the combination of zinc losses in the urine and in the sweat (Beisel, 1976).

Recently, research has indicated that stimulated leukocytes were capable of inducing fever (Powanda, 1979) and also decreasing serum zinc and iron concentrations (Pekarek and Beisel, 1971). This caused a rapid redistribution of zinc and iron to the tissues and especially a significant uptake by the liver (Pekarek et al., 1972). In addition, there was an acute increase in serum plasma globulins (Eddington et al., 1971) and a release of neutrophils from the bone marrow (Kambschmidt et al., 1973). When this leukocytic mediator was administered intravenously to Rhesus monkeys, it resulted in hypotension, tachcardia, vasodilatation, hypoalbuminuria and hypoproteinemia, but in these experiments fever was not present (Liu et al., 1979). The substance responsible for these biological effects appeared to be a complex proteinaceous material with a molecular weight ranging from 10,000 to 30,000 daltons (Pekarek and Beisel, 1971).

Powanda (1979) designated this substance that is capable of inducing fever as an "endogenous pyrogen" (EP) or "leukocytic endogenous mediator" (LEM). The LEM is responsible for inducing fever and other metabolic and physiologic alterations. Mapes and Sobocinski (1977) demonstrated that EP and LEM differed in their ability to cause release of rabbit peritoneal neutrophils *in vitro* where different concentrations of potassium ions were added. Concentrations of potassium greater than 5 mM inhibited EP release, whereas concentrations above 15 mM of potassium ions caused significant release of LEM.

Mapes et al. (1977) demonstrated a possible relationship between prostaglandins and LEM. The production of LEM by activated neutrophils was stimulated by adding 2 μM of prostaglandin E and F. Later, the addition of 2 μM of zinc ions caused a complete inhibition of LEM synthesis in *in vitro* stimulated neutrophils. However, the same

mechanism was not confirmed *in vivo* (Mapes et al., 1978).

Zinc and Wound Healing

In 1960, Strain et al. demonstrated that a mixture of methionine and zinc oxide increased the rate of wound healing in normal rats. The same results were not observed when each component was given separately. Seven years later, Pories et al. (1967) reported that a single dose of zinc sulfate accelerated the rate of healing after a pilonidal cystectomy in man. In the same year, Miller et al. (1967) confirmed that the rate of wound healing was slowed in zinc-deficient calves when compared to calves fed adequate zinc. They also indicated that two forms of zinc (zinc oxide and sulfate) did not differ in their effects on wound healing. Previously, an important observation was made by Miller et al. (1965) that the zone of parakeratosis which was present around the wounds of zinc-deficient calves was due to trauma and that the distribution of lesions in zinc-deficient calves was related to external or secondary factors such as mechanical trauma.

Sanstead et al. (1970) postulated that the impairment of wound healing and reduced tensile strength was related to the role of zinc in protein synthesis inasmuch as in zinc-deficient and pair-fed rats the region of the healing incision had fewer fibroblasts and a lower density of collagen. On the other hand, an increase in the uptake of ^{65}Zn in wounds of normal rats in the acute phase of healing coincided with an increase in vascularity. This increase was not present in skin of rats fed zinc (Savlov et al., 1962).

Zinc and Immunity

The immune system involves an interaction between many cells. At least two types of lymphocytes have been implicated in cellular immunity and in hypersensitivity. The major responses have been referred to as (1) humoral response, mediated by antibodies secreted by B-cells and (2) cellular responses, mediated by T-cells and macrophages.

A pronounced involution of the thymus has been reported in recent years in zinc-deficient rats (Quaterman, 1974), pigs (Whitenack et al., 1978) and mice (Fraker et al., 1977).

The importance of zinc and the thymus gland in maintaining the normal mechanism of the cell-mediated immune response has been demonstrated in zinc-deficient rats (Gross et al., 1979a) and man (Pekarek et al., 1979). In calves (Brummerstedt et al., 1974) and in man (Endre et al., 1975), genetic disorders which led to low zinc levels in the serum were responsible for a decrease in the immune response. When zinc was added to the diet, the immune function was reestablished.

The results obtained by Luecke et al. (1978) demonstrated that when A/J zinc-deficient mice were challenged with sheep red blood cells, there was no effect on the humoral immune response. However, when the deficiency was severe, the inanition impaired the thymus, which contributed significantly to the loss of immunity.

Pekarek et al. (1977) reported that *in vitro* lymphocytic response to phytohemagglutinin stimulation was impaired in zinc-deficient rats. The addition of levamisole to *in vitro* zinc-deprived lymphocytes improved their responses to phytohemagglutinin by 54%. No effect was observed in control lymphocyte cultures (Gross et al., 1979b).

Recently, DePasquale-Jardieu and Fraker (1979) postulated that because adrenal hypertrophy was present in zinc-deficient A/J mice, increased adrenal steroids may be responsible for the partial thymic atrophy and the subsequent impairment of the immune response in these animals. Their results indicated higher glucocorticoid values in zinc-deficient mice, but the function of T-lymphocyte cells had decreased 60% before the rise in plasma corticosteroid values. However, a reduction in the number of T-lymphocytes occurred 4 days after the initial rise of corticosteroid values. They suggested that the higher concentrations of corticosteroids may contribute to reduced immunocapacity of zinc-deficient mice.

Zinc Deficiency in Poultry

O'Dell and Savage (1957) at the University of Missouri and Roberson and Schaible (1958) at Michigan State University reported that chicks require zinc as a nutrient for normal growth as well as for feathering. The chicks were fed a ration containing less than 10 ppm zinc and grew slowly, failed to feather properly and developed a severe dermatitis of the foot.

Gross Pathology

During a 56-day experiment, the poor feathering in chicks was characterized by a weak and small rachis that lacked barbs. However, feathering problems were not observed when the chicks were fed a low zinc ration and were confined in galvanized brooders (Edwards et al., 1958). On the other hand, when chicks were fed a zinc-deficient ration for 3 weeks and housed in epoxy coated Petersime brooders, they had severe skin lesions (Young et al., 1958). In addition to skin lesions in zinc deficiency, the feathers had a tendency to be frizzly and stand

out from the body (O'Dell et al., 1958). A feather condition known as blister of the shaft was observed on poorly feathering chickens while they were housed in floor pens with shaving for litter and fed a ration containing 38 to 44 ppm zinc (Sunde, 1972).

In turkeys, Slinger et al. (1956) reported that foot dermatitis was associated with a zinc-deficient ration. Abnormal feathering was also observed in Japanese quail (Vohra, 1971) and ring-necked pheasants (Scott et al., 1959) when they were fed a ration with no zinc supplementation.

Histopathology

Dermatosis related to zinc deficiency in chickens has not been clearly described in the literature. In the initial research on zinc deficiency in chickens, a hyperkeratosis and mild thickening of the epidermis were observed in the skin. O'Dell et al. (1958) mentioned that the lesions were more pronounced in the skin of the wings, legs and feet. The epidermis was thickened, but no significant increase of mitotic figures in the basal layer was mentioned. The keratinized layers of the epidermis were increased in thickness. This condition was prominent on the shanks and feet. The hyperkeratinization of the skin extended into the feather follicles and resulted in atrophy of follicles and feathers. In some places the follicles were replaced by an inflammatory reaction. Wight and Dewar (1976) in Scotland observed that ducks fed a zinc-deficient ration after hatching developed severe lesions of the pedal epidermis and upper respiratory and digestive tracts. No distinction between the cells of the stratum basale and spinosum were observed. While epithelial atrophy occurred in early examples of the deficiency, acanthosis, hyperkeratosis, dyskeratosis

and heterophilic infiltration were characteristic of most cases. Erosion of the epidermis of the skin with the formation of crusts containing a secondary bacterial reaction was present and was followed by inflammatory reaction of the dermis.

Zinc Deficiency in Cattle

Miller and Miller (1962) at the University of Georgia established an experimental zinc deficiency in dairy calves. The first lesions were a rough and dry hair coat. Later, alopecia was present and started in the rear legs. The skin was thick, scaly and cracked, and deep fissures developed around the nose, ears, eyes and mouth (Ott et al., 1965). Two years later, Mills et al. (1967) in Scotland suggested that noticeable defects in the development of hooves and horns in calves were related to low zinc levels in the ration.

Under natural conditions, severe parakeratosis was described by Legg and Sears (1960) in cattle grazing in the Barbice savannahs area, Republic of Guyana. Later, reports from Finland (Haaranen and Hyppola, 1961; Haaranen, 1962) indicated that eczematous lesions in dairy cattle were associated with feeding low levels of zinc.

Histopathology

The skin of zinc-deficient calves had prominent acanthosis with elongation of the rete pegs. Miller and Miller (1962) mentioned that excessive keratin formation and the retention of nuclei were seen in the affected areas. Intercellular edema of the epidermis was also present. When the clinical signs became apparent, acanthosis was severe in the skin samples obtained above the hoof. The stratum granulosum layer contained a lesser number of cells than sections from zinc-supplemented calves.

Zinc Deficiency in Swine

According to Kernkamp and Ferrin (1953) at the University of Minnesota, a parakeratotic condition of an unknown etiology was a serious problem in young pigs 8 to 19 weeks of age. Most commonly, the hairless areas of the body were involved, such as the ventral aspects of the abdomen, flank or lower areas of the legs.

At first, the lesions consisted of erythematous areas that appeared in skin of the ventral and ventrolateral aspects of the body and medial surface of the thigh and then developed into well circumscribed papules 3 to 5 mm in diameter. The keratinous crusts (5 to 7 mm in diameter) that were not firmly adherent to underlying cutaneous structures were separated by fissures that were filled with a dark-colored, moist and sticky mixture of exudate and debris (Kernkamp et al., 1955).

According to Tucker and Salmon (1955), the syndrome previously described as "swine dermatitis" was associated with low levels of zinc in the ration.

A further description of parakeratosis was given by Voelker (1970). He emphasized that the erythematous condition appeared primarily in the perianal region and subsequently spread to the whole body. The skin became scaly, crusted and an oozing serosanguineous exudate was observed frequently. The distribution of the lesions appeared to be limited to the areas of the skin which were particularly vulnerable to mechanical trauma.

Histopathology

In their first description (Kernkamp and Ferrin, 1953) of parakeratosis, the crusts were described as composed of irregular hyperkeratotic stratum corneum having intact nuclei. Thickening of all of

the epidermal layers, except the stratum lucidum, and elongated rete pegs were described by Kernkamp et al. (1955). In further work, Anderson et al. (1967) described the same changes caused by zinc deficiency in swine, but they emphasized that the parakeratotic layers were not uniform and were more concentrated around the formation of the rete pegs. The dermal papillae were also elongated, and advanced lesions had a considerable degree of edema. The dermis also had an increased vascularity.

In more detailed research, Voelker (1970) mentioned that severe parakeratosis was the most prominent change seen in zinc deficiency and was especially severe in areas around the hair follicles. Keratin appeared to be more eosinophilic in the affected areas than in the areas free of dermatitis. In the most severely affected areas, the crusts were filled with large numbers of neutrophils and bacteria. Large globular eosinophili- inclusions were also present within the parakeratotic layers. These were usually found in sections taken from the feet. Acanthosis was a major component of the lesions. The rete pegs were also elongated and tended to anastomose with the adjacent ones. At the periphery of the affected areas, the spongiform spaces frequently became filled with neutrophils to form microabscesses, which resulted in disruption of the upper portion of the epidermis. Extracepillary erythrocytes and inflammatory cells were present, and the dermal papillae were dilated and edematous. The endothelial cells were swollen and hyperplastic in the tortuous microvasculature of the dermis. In the perivascular area, there was an accumulation of inflammatory cells and active fibroblasts. There was also an increase in the activity of the glandular epithelium of the apocrine sweat glands. The cells were basophilic and were a type of pseudostratified columnar epithelium.

Anderson et al. (1967) observed more sulfhydryl and less disulfite in the layers of parakeratotic tissue. Subsequently, an increase in both sulfated and unsulfated mucopolysaccharides was present in the dermis. Later, PAS staining indicated an increased amount of glycogen in the capillary endothelium of the dermal papillae and in the cells of the stratum spinosum.

Zinc Deficiency in Other Animals

Rats

According to Todd et al. (1934) at Wisconsin, subminimal levels of zinc in rat rations caused a loss of hair around the neck and shoulders. In extreme cases, there was complete shedding over the ventral side of the body. Stirn et al. (1935) observed changes in color from black to gray in rats fed a zinc-deficient ration. Occasionally, a slight reddening and encrustations around the eyes, nose and feet were present. Follis et al. (1941) in a detailed study observed that rats fed zinc-deficient rations had the same lesions mentioned by Wisconsin workers.

Follis et al. (1941) described the first histologic changes of zinc deficiency in rats which appeared at 33 days and consisted of hyperkeratosis. The epidermis was thickened and consisted of approximately 8 to 10 layers. The epithelial lining of the hair follicles became keratinized. Following this, the cells of the basal layers as well as those above it developed hydropic degeneration. Subsequently, edema of the epidermis was observed. Later, the crusted epidermis consisted of keratinized fibers, bacteria and leukocytes. In areas from which the hair had been lost, the follicles disappeared and a few mononuclear cells marked their previous location.

No substantial edema was observed in the dermis; however, neutrophils as well as monocytes were present. A decrease of keratohyalin granules in the epidermis was observed by Alvares and Mayer (1968).

Mice

During the first 2 to 3 weeks, zinc-deficient mice had a greasy hair coat with subsequent alopecia (Day and Skidmore, 1947). In a more recent study, Beach et al. (1980) fed 4 different levels of zinc to young mice and observed a variety of lesions in the zinc-deficient mice, including alopecia and extensive dermatitis.

Dogs and Cats

When dogs were fed a ration low in zinc with an excess of calcium, superficial skin lesions developed on the posterior aspect of the abdomen and rear legs (Robertson and Burns, 1963). Exfoliated areas from 10 to 20 mm in diameter were also observed. Kane et al. (1980) mentioned that kittens fed zinc-deficient rations had poor coats, characterized by thinning and slow hair growth. Scaliness of the skin and ulcerations of the buccal margins were also present.

Guinea Pigs

When guinea pigs were fed a zinc-deficient ration, they had rough hair coats and a scaly dermatitis of the feet and mouth (McBean et al., 1974). These lesions were not observed in guinea pigs fed a zinc-adequate ration.

Rabbits

According to Shaw et al. (1974), rabbits had clinical signs of zinc deficiency in the form of achromotrichia and some degree of dermatosis with hair loss. The lesions were not observed in all of the rabbits.

Monkeys

A variety of degrees of alopecia were observed in some squirrel monkeys by Macapinlac et al. (1967), but later Sandstead et al. (1978) reported that zinc-deficient pregnant Rhesus monkeys also developed alopecia on the ventral aspect of the abdomen and face followed by dermatitis. Recently, Swenerton and Hurley (1980) studied the effect of zinc deficiency on reproduction in Rhesus and Bonnet monkeys and confirmed earlier results.

Sheep and Goats

The first clinical signs of zinc deprivation in lambs were described by Ott et al. (1964) at Purdue University. By the fourth week of the deficiency, the skin areas, which were losing wool, became slightly red and wrinkled. Crusts developed on the ventral aspects of the body and the skin was severely damaged when exposed to cold. Histopathologically, the changes in the skin consisted of parakeratosis and acanthosis with elongation of the rete pegs. The lambs became more healthy after feeding phytic acid in their rations, but 5 weeks was not enough for the stratum germinativum to return to normal. In Colorado, lesions resembling those of zinc deficiency were reported in grazing sheep by Pierson (1966).

In 1964, Miller et al. at the University of Georgia observed that when goats were fed zinc-deficient rations they had clinical signs consisting of dull, rough hair coat, alopecia and cornifying skin, especially on the rear legs, neck, head, mouth, and scrotum. When the deficiency was severe, breaks and fissures were present on feet which later ulcerated, and horny overgrowth of the dental pads was present. Histopathologic examination revealed parakeratosis to be the most important change.

Streptotrichosis

Van Saceghan (1915) was the first to identify and name the agent of streptotrichosis, *Dermatophilus congolensis,* in cattle in Zaire. In 1934, he named it *Tetragenus congolensis.* Prior to this, Bull (1929) in Australia recovered a similar microorganism from "lumpy wool" disease of sheep and named it *Actinomyces dermatonomus.* Subsequently, in Scotland, Thompson and Bisset (1957) isolated a microorganism from "strawberry foot rot" disease of sheep and identified it as *Polysepta pedis.* Austwish (1958) confirmed that these species had the same genus but divided them into three species: *D. congolensis,* the agent of "streptothrichosis"; *D. dermatonous,* the agent of "lumpy wool"; and *D. pedis,* the agent of "strawberry foot rot." In a comparative study of the strains, Gordon (1964) concluded that all of these species were really varieties of a single species, *Dermatophilus congolensis* Van Saceghan, 1915.

Natural infections with *Dermatophilus congolensis* have been reported in cattle (Pier et al., 1963), sheep (Roberts, 1965), horses (Kaplan and Johnston, 1966), goats (Hudson, 1936), cats (Baker et al., 1972), owl monkeys (King et al., 1971), deer (Salkin et al., 1975), cottontail rabbits (Shotts et al., 1970), polar bears (Newman et al., 1975), raccoons (Salkin and Kistner, 1976), lizards (Montali et al., 1975), and man (Albrecht et al., 1974). Rabbits, mice and guinea pigs have been successfully infected experimentally by Roberts (1965).

The life cycle is not well elucidated, although in the initial stages the cocci enlarge and quickly form. The hyphae form branches and at the same time transverse septa are present (Bida and Dennis, 1976). According to Thompson and Bisset (1957), the filaments break off and release a motile and infective form of the organism, the zoospore (Bida and Dennis, 1976).

Transmission by direct contact, by the house fly (*Musca domestica*), and stable fly (*Stomoxys calcitrans*) was experimentally observed by Richard and Pier (1966). Ticks of the genera *Amblyomma, Boophilus, Hemaphysalis, Hyalomma, Ixodes* and *Rhpicephalus* have also been incriminated as potential vectors (Vandemaele, 1961).

Characteristics of the Pathogen

Dermatophilus congolensis is a gram-positive, pleomorphic bacterium which has been seen in culture medium in the form of filaments and cocci. The pathogen grows well on brain-heart infusion and on blood agar at 37 C in an atmosphere of 10 to 20% CO_2 for 48 to 72 hours (Gordon, 1964). The filamentous branching of *D. congolensis* was confirmed by electron microscopy (Gordon and Edwards, 1963).

Several studies have indicated that antigenic characteristics among *D. congolensis* strains, as measured either naturally or experimentally, are the same (Richard et al., 1976). However, Bida and Kelly (1976) mentioned that although precipitin and agglutinin antibodies are produced in *D. congolensis* infection, laboratory animals are not protected against reinfection by the same organism.

Pathogenesis

Under field conditions, infection by *D. congolensis* has been observed during wet periods and when there is injury to the skin (Roberts, 1967). After the cocci gain entrance to the skin from injury, they begin to germinate (Roberts, 1963). Filaments then penetrate the epidermis and invade hair follicles. The infection is superficial and confined to the upper epidermis, especially the stratum corneum and sometimes the dermo-epidermal junction (Roberts, 1965). Hyphae and coccoid forms are also observed in hair papillae and sebaceous glands (Amariki, 1974).

Pathological Changes

According to Oduye (1975), a marked erythema and edema are observed 24 hours postinfection. In addition, small vesicles are detected in some areas, followed by papules or pustules. A serous exudate is observed within 48 hours, with multiple raised areas in which the hair is matted. These scabs are small, but at 96 hours scab formations are seen in different areas and the small scabs start to form single large ones. When the crusts are removed, hemorrhage is present in some of the lesions. The hair growth is apparently normal at the sites where the scabs fall off or are removed. In contrast, the crusts result in a gelatinous material rather than a hard crust when kept moist by rain (Hart, 1976). These lesions, when generalized and severe, have contributed to death by restricting the movement of the affected animal and finally predisposing it to other infections, especially cutaneous myiasis (Wilkinson, 1979).

In horses, the lesions are similar to those seen in cattle. When rabbits were infected experimentally with organisms of equine origin, some of them developed discrete lesions (Kaplan and Johnston, 1966).

The histological changes that have been reported in both natural and experimental infections in domestic and wild animals were confined to the epidermis and consisted of acanthosis and hyperkeratosis with infiltrating inflammatory cellular debris. In areas from which scabs had fallen, a parakeratotic reaction was present. The branching hyphae of *D. congolensis* involve several hair follicles and the cornified epithelium of the external hair sheath. Acanthosis is also seen in the hair follicles. Intraepidermal microabscesses are commonly observed, especially close to or near the hair follicle (Oduye, 1976).

The hyphae do not usually invade the dermis, except when the hair follicle has become eroded and microabscesses develop (Bridges and Romane, 1961). Occasionally, the infected epidermis breaks and a marked neutrophilic reaction occurs beneath the epidermis. The epidermal cells in the stratum granulosum are disrupted and separated from each other by severe edema. The dermal exudate contains lymphocytes, macrophages , fibroblasts, and rarely multinucleated giant cells (Oduye, 1976). In sheep and rabbits experimentally infected by *D. congolensis,* the lesions were essentially the same as those reported by Roberts (1965).

Porcine Exudative Epidermitis

Porcine exudative epidermitis ("greasy pig disease") is a generalized dermatitis in pigs during their first month of life. It is characterized by a sudden onset, short duration, severe hyperhidrosis, excessive sebum secretion, desquamation of the epithelial cells and exudation.

Diseases similar to exudative epidermitis have been called necrotic dermatitis, pustular dermatitis, infectious dermatitis, exfoliative dermatitis, eczema, seborrhea oleosa, impetigo contagiosa suis and greasy pig disease (Underdahl and Twiehaus, 1975).

The lesions of porcine exudative epidermitis were reported to be caused by a cocci-like microorganism by Sampolinsky (1953), Jones (1956), Underdahl et al. (1965), L'Ecuyer and Jericho (1966) and Mebus et al. (1968). Other workers classified this microorganism as *Micrococcus hycus* (Sampolinsky, 1953; L'Ecuyer, 1966), *M. epidermidis* (Jones, 1956) and *Staphylococcus hyos* (Underdahl et al., 1965; Mebus et al., 1968).

Based on serological studies of the etiological agent of greasy pig disease, Hunter et al. (1970) concluded that there were 2 strains. The first was a nonpathogenic strain B of staphylococci which was frequently found in the nose and on the skin of both healthy and infected pigs. The second, strain A, was isolated only from those pigs which had been in contact with the infection. According to the 8th (1974) edition of *Bergey's Manual of Determinative Bacteriology*, the microorganism was identified as *Staphylococcus epidermidis* biotype 2, which recently was redescribed as *S. hycus* by DeVriese (1977).

Underdahl and Twiehaus (1975) emphasized that the barrier of the skin must be broken in order for infection to occur. Young pigs could be infected through open wounds caused by bites or abrasions that usually occur on the knees and feet. In addition, they concluded that a large number of pigs had contracted the infection when they were in contact with pigs that had also been infected by a vesicular viral infection. This conclusion was also supported by earlier research of Underdahl et al. (1963) and Obel (1968).

Kernkamp et al. (1955) mentioned that different types of microorganisms had been isolated from parakeratotic pigs. Jones (1956) and Ristic et al. (1956) suggested that exudative epidermitis was probably primarily associated with an inadequate ration of the animals. This idea was documented by experimental data which indicated that a well balanced ration may decrease the susceptibility to bacterial and parasitic infections and at the same time increase the susceptibility to viral infections (Scrimshaw et al., 1959). Later, Whitehair and Miller (1975) also mentioned that well nourished swine have greater resistance to infection than those poorly nourished.

Experimentally, the infection was transmitted by different routes. Underdahl et al. (1963), using young pigs obtained by hysterectomy, inoculated them intranasally, orally or subcutaneously and irrigated areas like the eyes, ears, and skin. Pigs which had had contact with infected pigs had more severe lesions and a higher mortality rate than those exposed by other routes. Later, L'Ecuyer (1966) reported that intravenous inoculation and scarification were also as effective as the techniques used by Underdahl et al. (1963).

Jones (1956) was the first to describe the pathologic aspects of naturally occurring disease in Iowa. In his opinion, the course of the disease may follow three different forms: peracute, acute and subacute. The peracute form is characterized by a sticky and greasy exudate that consists mainly of sebum, serum and sweat. It becomes generalized and involves the entire body surface (L'Ecuyer, 1966; Mebus et al., 1968). Cutaneous erythema is pronounced and a thick line of gummy exudate forms at the mucocutaneous junction. The production of this exudate ceases temporarily and subsequently a thin, dry scab forms in areas where the hair was thin. When the scabs are removed, a highly inflamed skin is exposed. The skin beneath these areas has an accumulation of a wax-like layer that has a rancid odor (Ristic et al., 1956). The acute form is similar to the peracute form, but the infection is less severe, even though the skin becomes more thickened and wrinkled. Brown skin spots increase in number and size. Small vesicles and pustules rupture and form ulcers. The skin remains "greasy." Scabs and crusts break off and form deep fissures. In the subacute form, the lesions are the same as those of the acute form, but the skin is more severely affected.

Histopathology

The histopathology of naturally infected pigs is described in detail by Jones (1956). The brown spots seen on gross examination consist mainly of sebum mixed with cellular debris, bacteria and dirt. Brown spots are also observed at the openings of sweat glands and contain sweat instead of sebum. The epidermis is slightly hypertrophic and is characterized by enlarged rete pegs and an increase in number of cells of the stratum spinosum. The nuclei of the stratum granulosum are more visible than in the normal skin. Parakeratosis is indicated by an increase of the stratum corneum and retention of nuclei. As the disease progresses, there are focal areas of inqer- and intracellular edema that later result in hydropic degeneration of the stratum spinosum. The stratum corneum becomes thicker, acquires more cracks and crevices develop, with an accumulation of cellular debris and microorganisms. Inflammatory cells, mainly neutrophils and occasionally lymphocytes, migrate throughout the epidermis. At the same time, vesicles and pustules form and later rupture. During this time, a marked hyperemia occurs in the dermis. The lymphatic vessels dilate. There is a moderate increase of inflammatory cells, principally eosinophils. All sweat glands are distended and active. These secreting epithelial cells are swollen. The sebaceous glands are recognized by their activity and the material present in the lumen.

The inflammatory process occurs initially in the part above the point where the sebaceous glands had their follicles. When the reaction becomes more severe, the whole follicle becomes involved as well as the sebaceous gland and the sweat gland (Jones, 1956).

In experimentally infected pigs the lesions were similar to those described for naturally occurring infection (L'Ecuyer and Jericho, 1966; Mebus et al., 1968).

Histochemical studies of the cutaneous lesions were conducted by Obel (1968). A PAS-positive material accumulated in the stratum granulosum. The keratohyaline granules disappeared and parakeratotic layers containing abundant protein-bound sulfhydryl groups and lipids. Acid phosphatase and succinic dehydrogenase were seen in the stratum spinosum and alkaline phosphatase was present in the stratum basale.

Summary

The skin is an important barrier between the animal and its environment. It consists of two important structures. The outer layer, or epidermis, is responsible for protecting the body from physical, chemical and biological injuries. Regional differences of the epidermis represent adaptations to a particular function. The inner layer, the dermis or corion, supplies nutrients to the epidermis. The appendages of the skin vary according to the species involved. The appendages of the skin also protect and regulate the temperature on the surface of the body.

Changes in the skin are associated with localized and systemic disorders caused by nutritional deficiencies, infections, environmental factors and endocrine dysfunctions. These changes may affect the integrity of the whole skin or some of its components.

Experimentally, zinc deficiency produces skin lesions in a variety of animals. In swine it is characterized by a severe dermatosis with hard, dry, crusted proliferation of the superficial epidermis that involves especially the ventral aspects of the body. In cattle, the lesions are mostly restricted to the lower jaw, lateral

aspects of the neck, and the hocks. In chicks, the lesions consist of poor feathering, failure of growth, and dermatitis of the foot. Microscopically, in these species there is parakeratosis, hyperkeratosis and acanthosis with subsequent dermal infection.

In addition to having a role in maintaining the integrity of the skin, zinc also has an important role in the development of immunity to infection. During an acute process the leukocytic mediators are involved in sequestering serum zinc to increase protein synthesis by the liver. This results in stimulation of the body defense against an infectious process.

In summary, zinc has an important role in maintaining optimum health of man and animals. Additional research is therefore warranted, especially on the role of zinc in maintaining a healthy skin in resistance to a variety of skin diseases in livestock that impair efficient production and decrease the values of the hides.

OBJECTIVES

The objectives of this research were:

1. To determine the gross and histologic morphology of the skin of normal calves, chicks and pigs.

2. To investigate and compare the pathologic changes in the skin of zinc-deficient calves, pigs and chicks.

3. To evaluate the susceptibility and the lesions of the skin of calves when exposed to *Dermatophilus congolensis* infection.

4. To determine the susceptibility and the lesions of the skin of zinc-deficient pigs when exposed to *Staphylococcus hycus* infection.

MATERIALS AND METHODS

General

Integumentary tissue, especially skin, used for this research was collected from calves, chicks and pigs. It was collected during experiments in progress in which these animals were fed either zinc-deficient or zinc-adequate rations. Integumentary tissue was also collected from animals fed zinc-deficient or zinc-adequate rations and exposed or unexposed to specific skin infections. These experiments involved young animals and in most instances the research was initiated when the animals were a few days of age. The research was conducted during the years 1978 to 1980. The animals were maintained at the Veterinary Research Facilities (Barn F), Poultry Science Laboratories, and the Swine Farm. Tissue analyses were conducted mainly in the Animal Husbandry and Pathology Laboratories. The tissues used in this research were from experiments done in cooperation with Dr. A. P. Telles and Dr. E. R. Miller on the general pathology of zinc deficiency in calves and swine and with Dr. A. L. Caetano and Dr. R. K. Ringer on the pathology of zinc deficiency and susceptibility to infectious bursal disease virus (EBDV) in chicks. A semipurified type of ration that was low in zinc was fed in all experiments. The low-zinc mineral mixture used was as described by Miller et al. (1968) and was used successfully to produce a zinc deficiency in previous swine experiments (Voelker, 1970; Whitenack et al., 1978). The low-zinc mineral mixture used was prepared by Dr. E. R. Miller. The

soy-protein component of the ration also contributed a variable amount of zinc. In the pig and chick, the phytic acid in soy-protein chelates zinc and contributes to its unavailability. However, in the ruminant the microflora interferes with phytic acid chelation and thus more zinc would be available to the animal. Therefore, in the calf experiments a source of protein other than soy-protein was used initially. The protein used was egg-white protein, which was used successfully to produce zinc deficiency in rats and mice. Other components of the ration would not be expected to contribute appreciably to the zinc supply. In all experiments a special effort was made to maintain the animals in facilities and with feeding and watering equipment that would minimize exposure to zinc.

Collection of Tissue Samples

Blood

Blood from the calves, pigs and chicks was collected into tubes containing ethylenediaminetetraacetic acid (EDTA) at selected intervals, during the course of the experiments. Samples were also collected without anticoagulant and were allowed to clot in zinc-free plastic tubes. The separated serum was frozen at -24 C and used later for zinc analyses and protein electrophoresis.

Skin

A round dermal medical punch,[a] 0.9 mm in diameter, was used to obtain skin samples in the calves and pigs. These samples were

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[a]Lansing Medical Supply Co., Lansing, MI.

collected at selected intervals during the course of the experiments. A subcutaneous injection of 4 ml of procaine hydrochloride was used as a local anesthetic. The specimens were fixed in 10% buffered formalin.

Hair

The hair samples from the calves were collected from the lateral aspects of the neck with electric clippers. The samples were stored in plastic bags for zinc analyses.

Feathers

Feathers were removed from the wings and from the dorsal midline of the body of the chicks at necropsy. The samples were stored in plastic bags until zinc analyses were performed.

General Laboratory Analyses

Hematology

The numbers of white blood cells per cubic millimeter were counted in a hemacytometer. Blood smears were stained with Wright's stain and differential leukocytic counts made. Packed cell volume was determined using microhematocrit tubes. Hemoglobin was evaluated by a cyanmethemoglobin standard and determined using a spectrophotometer.[b]

Serum Protein Electrophoresis

Serum was applied to cellulose acetate plates and separated by electrophoresis for 15 minutes at 180 volts. The plates were then

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[b]Linear Absorbance Spectrophotometer, Perkin-Elmer, Norwalk, CT.

stained with Ponceau[c] stain. Subsequently, the strips were fixed in methanol for 3 minutes. The plates were then dried in an oven at 50 to 60 C for 6 minutes. The values were determined by using a densitometer[c] and were expressed in g/dl.

Histopathologic Techniques

Tissues, after being fixed in 10% buffered formalin, were processed by an automatic processer,[d] embedded in paraffin, and sectioned at 5-6 µm. The sections were stained with hematoxylin-eosin and other special stains were used where appropriate (Luna, 1968). Frozen sections of the skin were cut in a cryostat and stained for succinic dehydrogenase enzymes according to Thompson and Hunt (1966).

Zinc Analysis

An atomic absorption spectrophotometer was used to determine zinc in samples of serum, hair, feathers and feed. The analysis was performed in the 213.9 nm resonance line and employed the standard condition for zinc analysis according to published analytical methods (Perkin-Elmer, 1974). The values are expressed in µg/ml for serum samples and µg/g for feed, feathers and hair. The zinc content of serum was determined according to the following technique. One milliliter of serum and enough deionized water to make 10 ml were mixed and poured by automatic dilution into plastic tubes. The samples were then analyzed in the spectrophotometer.

A few grams of feathers or hair were washed in individual plastic bottles with screw caps. To each bottle, 150 ml of 2% soap[e] solution

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[c]Helena Laboratories, Beaumont, TX.

[d]Fisher Scientific Co., Livonia, MI.

[e]7X-O-Matic (non-ionic detergent), Limbro Chemical, New Haven, CT.

was added. The bottles were transferred to a mechanical shaker and the samples were mixed for 30 minutes. The samples were then rinsed with 1,000 ml of deionized water and placed in glass petri dishes to be dried in an oven at 120 C overnight. Dried samples of hair or feathers of about 500 mg each were transferred to 50 ml Erlenmeyer flasks. To each sample, 10 ml of concentrated HNO_3 and 1 ml of concentrated $HClO_4$ were added. The mixture was placed on a hot plate at 220 C inside a safety hood and allowed to completely digest. The digested samples were transferred to volumetric flasks and deionized water added to each sample to make 100 ml. Finally, the samples were analyzed.

Two grams of ration were taken from the samples. The samples were transferred to porcelain crucibles. They were then reduced to ash overnight at 340 C in an electric furnace. The ash was dissolved in 5 ml of 3 N HCl. The mixture was then allowed to react on an electric hot plate for 10 minutes. The resulting mixture was transferred to a volumetric flask and deionized water was added to make 100 ml. The samples were then analyzed.

Statistical Analyses

Data were statistically analyzed according to Gill (1978) and to the Statistical Package for Social Sciences (SPSS-Northern University) at the Michigan State University Computer Center.

Calf Experiments

A total of 20 neonatal calves from the Michigan State University Dairy Herd was used in 2 experiments. They were maintained in individual wooden pens with concrete floors. Plastic feeders and watering containers were used to avoid zinc contamination. Each calf was fed

colostrum for 2 days. The ration was then changed to whole milk and at about 21 days of age the ration was gradually changed to a zinc-deficient or zinc-supplemented ration.

In Experiment 1, the zinc-deficient ration was composed of glucose monohydrate (cerelose), egg-white protein, lard, a complete vitamin mixture, and the low-zinc, mineral mixture. This ration contained 10 to 11 ppm zinc. As the calves were changed from a milk ration to the experimental ration, diarrhea and inappetence occurred. For a period of 2 to 3 months, a variety of techniques were used to adjust the calves to the basal egg-white protein ration. These efforts were all unsuccesful, and any attempt to include the egg-white protein in the ration resulted in diarrhea and inappetence. It was concluded that calves would not tolerate this protein source. For the rest of the experiment soy-protein was used. The duration of the experiment was approximately 6 months.

In Experiment 2, the same general ration was fed and a mixture of soy-protein and lactalbumin was used as the protein. This ration contained 3.1 to 7.7 ppm zinc on the initial analysis. A later analysis was much higher in zinc, which was due to a higher content of zinc in a different source of lactalbumin. Apparently the zinc content of lactalbumin varies widely depending on the method of separating this protein in milk. The duration of the experiment was approximately 2 months.

Experimental Design

The initial general design of each experiment was as follows:

Experiment 1

Group 1 - Seven calves fed the basal zinc-deficient ration (10-11 ppm zinc)

Group 2 - Three calves fed the basal ration, supplemented orally with zinc to supply 150 mg zinc 3 times a week

Experiment 2

Group 1 - Six calves fed the basal zinc-deficient ration (3.1-7.7 ppm zinc)

Group 2 - Four calves fed the basal ration, supplemented orally with zinc to supply 200 mg daily.

In Experiment 1, at 2 weeks before termination of the zinc deficiency study, calves from each group were reallotted for exposure to *D. congolensis*. Three of the calves fed the zinc-deficient ration and one fed zinc orally were exposed to *D. congolensis*. One calf from each group was used as a control, not exposed to the pathogen.

In Experiment 2, at 1 week before termination of the zinc deficiency study, 3 zinc-deficient calves and 2 zinc-supplemented calves were exposed to *D. congolensis*. Three zinc-deficient and 2 zinc-supplemented calves were left as unexposed controls.

Clinical Records

Detailed records were maintained on the calves as to the general health, weight changes, behavior, and condition of the skin. The calves that had severe diarrhea and at times respiratory distress were given oxytetracycline tablets orally and penicillin-streptomycin intramuscularly.[f]

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[f]Combiotic, D-M Pharmaceuticals, Inc., Rockville, MD.

Exposure of the Skin to *D. congolensis*

The strain of *D. congolensis* used to expose the calves was isolated and identified by Dr. G. R. Carter. It was obtained from a clinical case of equine cutaneous streptotrichosis. The microorganisms was maintained in brain-heart infusion agar[g] as a stock culture and subcultures were maintained in trypticase soy agar with 5% bovine blood.[h]

The areas of the calf skin to be exposed to *D. congolensis* were clipped close to the surface with electric clippers. The area was then swabbed with xylene to remove fat. Scarification of the skin was made longitudinally and vertically with a 28-gauge needle. The areas were then swabbed with 24-hour-old cultures of 6×10^7 microorganisms/ml. In the same calf, control or reference sites were prepared in the same way and swabbed with sterile nutrient broth. The areas were examined in detail at 48-hour intervals.

Necropsy

The calves were immobilized with succinylcholine and then electrocuted. Skin samples were taken from different parts, such as: muzzle, lateral aspect of the neck and trunk, anterior and posterior fetlocks, hocks and skin lesions. Samples for Dr. Telles' research were taken from thymus, subcutaneous lymph nodes, lungs, liver, intestine, heart, skeletal muscle, brain, pancreas, salivary glands, thyroids, adrenals, bone and bone marrow.

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[g]Difco Laboratories, Detroit, MI.

[h]Bio-Quest, Cockeysville, MD.

Chick Experiments

Two experiments were conducted using a total of 148 one-day-old chicks. In Experiment 1, the chicks were housed in epoxy-coated galvanized batteries. The ration was composed of soy-protein, cerelose, corn oil, vitamins and a mineral mixture (with and without zinc). The zinc-deficient ration contained 12 ppm zinc and the control ration 48.4 ppm zinc. In the first experiment, typical clinical signs of zinc deficiency were not observed. There was, however, a lack of secondary feathers on the lateral aspect of the body and the foot pads were scaly. Suspecting that the chicks were not completely isolated from zinc contamination, the facilities were reevaluated. In the second experiment, the epoxy-coated galvanized batteries were replaced by plastic-coated steel cages. The water which was distilled in Experiment 1 was replaced by deionized water in the second experiment.

Experimental Design

The design of each experiment was:

Experiment 1

Group 1 - Twenty-seven chicks fed zinc-deficient ration (12.0 ppm zinc)

Group 2 - Twenty-seven chicks fed zinc-adequate ration (48.4 ppm zinc)

Experiment 2

Group 1 - Forty-seven chicks fed zinc-deficient ration (12.0 ppm zinc)

Group 2 - Twenty-three chicks fed zinc-adequate ration (48.4 ppm zinc).

Group 3 - Twenty-four chicks fed zinc-adequate ration and feed consumption limited to the same amount of feed as consumed by 24 zinc-deficient chicks.

In Experiment 2, when the zinc-deficient chicks had pronounced clinical signs of zinc deficiency, half of the chicks in each treatment were exposed to the infectious bursal disease virus. This virus was supplied by the National Animal Disease Center, Ames, IA.

Clinical Records

Records were maintained on the chicks as to weight changes, food consumption, and clinical signs. Characteristics of the skin and feathers were emphasized.

Necropsy

The chicks were killed by cervical dislocation. Skin samples were taken from different areas of the body, such as: comb, antero-lateral aspects of the body, wings, shanks, metatarsal foot pads and uropygial gland. Tissue samples were also taken from the tongue, esophagus, crop, intestine, pancreas, liver, lungs, kidneys, heart, skeletal muscle, brain, testicle, thymus, lymphatics, bone and bone marrow.

Pig Experiments

A total of 12 four-week-old pigs from 3 different litters from the Michigan State University Swine Herd was used. They were allowed to nurse their dam for approximately 4 weeks. They were then divided into 4 groups of 3 pigs each, as given in the experimental design, and maintained in slotted-bottom stainless steel cages, 3 pigs per cage, and fed their assigned experimental ration. The basal experimental ration was composed of soy-protein (30%), glucose monohydrate (50%),

cellulose (5%), a complete vitamin mixture, and the mineral mixture (with or without zinc). The deficient ration contained 9.4 ppm zinc, while the control ration contained 100 ppm zinc. At the time the pigs in groups 1 and 2 (6 pigs) developed parakeratosis, groups 2 and 4 were moved from the Swine Farm to Barn F of the Veterinary Research Farm to be exposed to *S. hycus* infection. The microorganism that was used to expose the pigs was obtained from a pig with clinical signs of "greasy pig disease" by Dr. A. L. Trapp. It was isolated and identified by Dr. G. R. Carter as *Staphylococcus hycus*.

Experimental Design

The general design of the experiment was:

Group 1 - Three pigs fed a zinc-deficient ration (9.4 ppm zinc)

Group 2 - Three pigs fed a zinc-deficient ration and exposed to *S. hycus* at the time that clinical symptoms of parakeratosis and blood zinc values were low

Group 3 - Three pigs fed the zinc-adequate ration (100 ppm zinc)

Group 4 - Three pigs fed the zinc-adequate ration and exposed to *S. hycus* at the time of clinical symptoms of parakeratosis in groups 1 and 2.

Clinical Records

Records were maintained on food consumption, weight changes and clinical signs, especially skin lesions. At the start of the experiment, all of the pigs were given orally neomycin[i] as a preventive of enteric infection.

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[i]Anchor Laboratories, Inc., St. Joseph, MD.

Exposure of the Skin to *S. hycus*

After clinical signs of zinc deficiency appeared in groups 1 and 2, pigs in groups 2 and 4 were exposed to *S. hycus* by swabbing the conjunctiva of the eyes and the lesions caused by zinc deficiency with 10^9 microorganisms/ml. As the infection did not appear in 5 days, a second exposure consisting of the same amount of *S. hycus* was given subcutaneously to these pigs.

Necropsy

The pigs were immobilized with succinylcholine and then electrocuted. Skin samples were taken from different areas of the body, such as: snout, ventral and lateral aspects of the neck and body, anterior and posterior fetlocks and posterior hocks and their respective coronary bands, tongue, esophagus, and finally the cardia of the stomach. Samples for Dr. Telles' research were taken from subcutaneous and mesenteric lymph nodes, thymus, lungs, liver, intestine, heart, skeletal muscle, brain, pancreas, thyroids, adrenals and bone.

RESULTS

Evidence of zinc deficiency was experimentally produced in calves, chicks and pigs. This thesis emphasizes the results of the research pertaining to the morphologic and pathologic features of the integumentary tissue, especially the skin. The results of zinc deficiency in other tissues and organs will be reported by Drs. Telles and Caetano. The description of changes in the integumentary system associated with zinc deficiency and specific infectious agents will be stressed as well on the important clinical signs and pertinent results of laboratory analyses associated with zinc deficiency in each species.

Zinc Deficiency and Skin Infection in Calves

Experiment 1

Although the egg-white protein is very low in zinc, it had an overall deleterious effect on the health of the calves in this experiment. The deleterious effect was manifested initially by a diarrhea which was followed by inappetence, weakness, dehydration, respiratory problems and eventually death in 4 of the 10 calves. One zinc-supplemented and 3 zinc-deficient calves died. The diarrhea occurred in all calves regardless of whether they were supplemented or unsupplemented with zinc. Attempts to avoid the deleterious effects of egg-white protein by different feeding regimens, dilution

of the egg-white and addition of fibrous material were unsuccessful. The experiment was completed by substituting soy-protein for egg-white. Although this improved performance, the calves still grew at a subnormal rate and had a stunted appearance. All the calves had a lower than normal growth rate in this experiment, but the calves unsupplemented with zinc manifested more clinical signs of zinc deficiency than the supplemented calves.

Experiment 2

A combination of lactalbumin and soy-protein was used as the low-zinc protein in this experiment. General performance of the calves was considered satisfactory. The zinc content of lactalbumin varied widely (10 to 60 ppm) depending on the technique used in separating the lactalbumin in milk. This information would suggest that the zinc content needs to be monitored carefully in using lactalbumin as a source of low zinc protein.

Clinical Signs

In both experiments the calves supplemented with zinc appeared to have a thick hair coat and the surface of the skin was covered by a thin film of sebum. In specialized areas, such as the muzzle, the surface was smooth and moist (Figure 1).

In both experiments the first clinical sign in the zinc-deficient calves was a decrease in daily feed consumption when compared to the amount consumed by the zinc-supplemented calves. Hypersalivation was noticed soon after the calves became anorectic. The hair coat soon appeared dry, scaly, dull and the hair was stiff. Alopecia appeared in areas free of dermatosis, such as the lateral aspects of the neck. The lesions, later, extended to the lower jaw, around the muzzle,

Figure 1. General appearance of a calf fed the basal ration supplemented with 150 mg zinc 3 times a week.

Figure 2. General appearance of a calf fed a zinc-deficient basal ration.

around the eyes and extremities of the legs (Figure 2). Later, the areas around the muzzle, mainly the anterior portion of the jaw, were covered by crusted material and an accumulation of feed and water (Figure 3). During this period, some of the calves appeared to have tenderness of the fetlocks. The skin of zinc-deficient calves was erythemic in areas of the body which were later affected by the dermatosis, particularly in the extremities of the legs. Subsequently, the surface of the fetlocks became dry, encrusted and was subject to injury (Figure 4). As the deficiency progressed, the lesions were more extensive. This was observed especially on the fetlocks. In the rear legs, lesions were also observed in the coronary bands, hocks and perianal region. Crusts were formed mainly because of accumulation of debris in areas of the skin severely affected by the dermatosis, such as the fetlocks and hocks.

In Experiment 2, skin lesions appeared in the calves fed the zinc-deficient ration approximately 4 weeks after the experiment started. The lesions then disappeared in about 2 weeks, and it was found that serum zinc levels had increased. This was due to a supply of lactalbumin that contained a higher content of zinc than the initial supply.

Laboratory Analysis

Zinc Values. Serum zinc values of the calves in both experiments are summarized in Table 1. The values listed are those at the termination of phase A of the zinc experiments (approximately 6 months for Experiment 1 and 2 months for Experiment 2) and at 2 weeks after *D. congolensis* infection in Experiment 1 and 1 week after infection in Experiment 2.

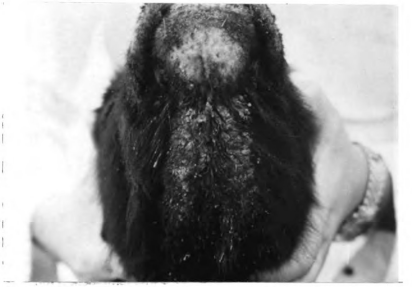

Figure 3. Gross appearance of the lower jaw of a calf fed a basal ration. Alopecia was apparent and accumulation of feed material adhered to the hair.

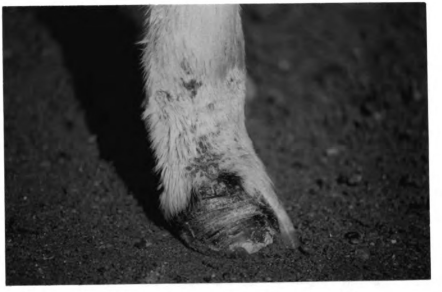

Figure 4. The rear foot of a calf fed a basal ration. Alopecia was apparent and crusted material was adhered to the skin.

Table 1. Serum zinc values (μg/ml) in calves at phase A in Experiments 1 and 2, and after D. congolensis infection

Treatments	Values[a]			
	Experiment 1		Experiment 2	
	Phase A	2 Wks After Inf.	Phase A	1 Wk After Inf.
Zinc-deficient ration	0.42±0.04[e](25)[d]	0.43±0.03 (18)	0.67±0.05 (18)	0.64±0.08 (9)[f]
Zinc-supplemented ration	0.43±0.03[b](13)	0.42±0.03[b](6)	1.07±0.04[c](12)	1.09±0.01[c](6)

[a]The values are expressed in mean ± SE.

[b]Supplemented with 150 mg zinc 3 times a week.

[c]Supplemented with 200 mg zinc daily.

[d]Figures in parentheses denote the number of samples.

[e]NS for treatments.

[f]Different ($p<0.0005$) compared with zinc-deficient ration, supplemented with zinc.

In Experiment 1, the calves fed the zinc-deficient ration had about the same serum zinc values as the calves supplemented with 150 mg zinc 3 times a week. Serum zinc values were not influenced by *D. congolensis* infection, although lesions of *D. congolensis* infection were present in the calves.

In Experiment 2, there were significant differences in serum zinc values between the calves fed the zinc-deficient ration and those fed the same ration and supplemented with 200 mg zinc daily. As in Experiment 1, infection with *D. congolensis* did not influence serum zinc values. The calves supplemented with zinc had a healthy skin, whereas the unsupplemented calves had an unhealthy skin and lower serum zinc values. Serum zinc values in the calves fed the zinc-deficient ration were higher in Experiment 2 when compared to calves in Experiment 1. Likewise, serum zinc values for calves supplemented with 200 mg zinc daily were higher in Experiment 2 than the calves supplemented with 150 mg zinc 3 times a week in Experiment 1. No differences occurred in the concentration of zinc in the hair of the calves supplemented or unsupplemented with zinc in Experiment 1.

Hematologic Findings. Hematologic results of calves in Experiment 1 (Appendix, Table A1) and Experiment 2 (Appendix, Table A2) are summarized. Hemoglobin and packed cell volume were not affected by treatment in either experiment. White blood cell values were in the normal range. However, they were low in the calf fed a zinc-adequate ration and exposed to *D. congolensis* in Experiment 1. The neutrophilic values were higher in calves fed the zinc-deficient ration and exposed to *D. congolensis* in Experiment 1 and were low in calves fed the zinc-supplemented ration and exposed to *D. congolensis* in Experiment 2.

The lymphocytic values were lower in calves fed the zinc-supplemented ration and exposed to *D. congolensis* in Experiment 1. No changes occurred in the eosinophilic, monocytic and basophilic values.

Serum Protein Electrophoresis. The protein electrophoretic values of calves in Experiments 1 and 2 are summarized in Appendix Tables A3 and A4.

Albumin values were low in calves fed a zinc-deficient ration and exposed to *D. congolensis* infection in both experiments. The β-globulin values were higher in calves fed the zinc-deficient ration and exposed to *D. congolensis* in Experiment 1. The γ-globulin values were low in calves supplemented with zinc and exposed to *D. congolensis* in both experiments.

Histopathology

The detailed microscopic changes of the integumentary tissues, which was the emphasis in this research, will be described for those calves in both experiments with the most pronounced clinical signs and gross lesions due to zinc deficiency or to zinc deficiency and skin infection.

Epidermis. The epidermis of the zinc-supplemented calves was relatively thin, except in areas of the muzzle and the hooves which were relatively thick. The stratum basale was organized in a row of columnar type cells. As they moved to the stratum spinosum, the keratinocytes became polyhedral in appearance (Figure 5). In the skin of the body, the stratum spinosum consisted of an irregular line of cells. In areas of the muzzle and hooves, the stratum spinosum was composed of 3 or more rows of polyhedral keratinocytes. The cells became flattened and were incorporated into the stratum granulosum, which was characterized by the presence of keratohyalin granules.

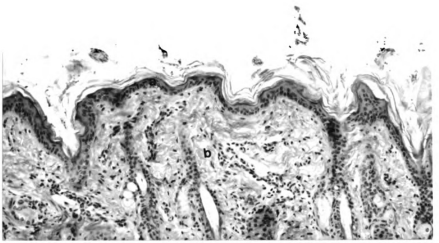

Figure 5. Microscopic appearance of the skin of a calf fed the zinc-supplemented ration (Experiment 1). Epidermis (a) and dermis (b). Hematoxylin and eosin; 120X.

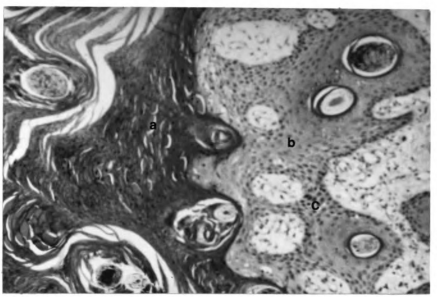

Figure 6. Microscopic appearance of the skin of a calf fed the zinc-deficient ration (Experiment 1). Parakeratosis of the stratum corneum (a), acanthosis of the stratum spinosum (b). Hematoxylin and eosin; 120X.

These granules were more abundant around the hair follicle as well as in areas of the muzzle. The stratum lucidum was a distinct area between the stratum corneum in areas of the hooves and horns, but in the rest of the body it was not distinct. On the surface of the stratum granulosum, the cells became anucleated and flattened to form the stratum corneum, which was relatively thick in areas of the hooves and horns.

In the zinc-deficient calves in areas where alopecia was more intense, biopsy of the skin was performed on the lateral aspect of the neck. The stratum corneum was somewhat thicker and in some areas was replaced by mounds of parakeratotic stratum corneum. As the reaction became severe, parakeratosis was observed throughout the stratum corneum, especially in areas of the extremities of the feet. In more affected areas, the crusted material consisted of several layers of parakeratotic keratin (Figure 6). In addition to severe parakeratosis, the skin of the fetlocks, pasterns, coronary bands and hocks contained many inflammatory cells and debris.

The granular layer was partially absent in lesions and was also replaced by spongiosis beneath the parakeratotic stratum corneum. Keratohyalin granules were absent in severely affected areas, except in areas around the hair follicles. Acanthosis was a major component in severely affected areas. In mildly affected areas, such as the lateral aspect of the neck, the skin was slightly thicker. Epidermal thickening increased as the deficiency intensified and the rete pegs became progressively elongated, extended deeper into the dermis and tended to anastomose with adjacent ones. The cells of the stratum basale were poorly differentiated. The keratinocytes were lined in a single row of columnar type cells. There was an increase in

intercellular space between cells, so that the intercellular bridges were prominent. Where the cells had moved towards the stratum spinosum, the keratinocytes were progressively swollen, leading to a severe spongiosis. The spongiform spaces became filled with either proteinaceous-like material or inflammatory cells to form a spongiform abscess which resulted in partial disruption of the epidermis.

Dermis. The dermis of the calves supplemented with zinc consisted of bundles of collagen fibers which were oriented horizontally and were characterized by immature fibroblasts mainly around blood vessels. Substantial papillary areas consisting of fine collagen fibers rested between the rete pegs in the dermis of the muzzle and in the cornified areas. The dermis had a moderate number of superficial capillaries which formed a plexus inside of the dermal papillae.

In calves fed zinc-deficient rations, the dermis beneath the areas affected by spongiosis was edematous. The blood vessels of the superior plexus were greatly dilated in areas affected by the inflammatory process. The endothelial cells of the blood vessels were somewhat enlarged. There were extravascular inflammatory cells, mainly neutrophils, in areas severely affected by the deficiency.

Appendages. In general, the hair coat of calves supplemented with zinc was thick. The epidermis of the hair follicle was thin and had the same structure as the epidermis. The sebaceous glands were in association with the hair follicle. The glandular area consisted of a small number of cells (Figure 7). The apocrine sweat glands were coiled glandular areas with sacculated appearing lumens and the glandular areas were lined with flattened cuboidal cells.

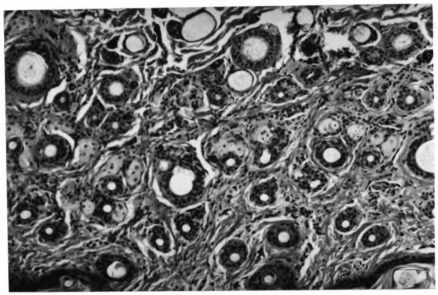

Figure 7. Sebaceous glands (arrow) of a calf supplemented with zinc (Experiment 1). Hematoxylin and eosin; 120X.

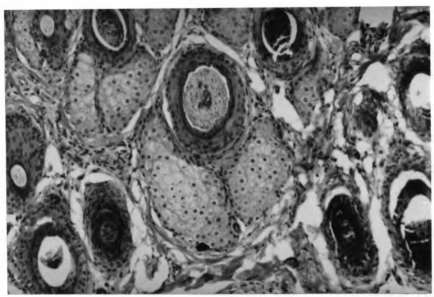

Figure 8. Hyperplastic sebaceous glands (arrow) in a calf fed the zinc-deficient ration (Experiment 1). Hematoxylin and eosin; 120X.

In calves fed the zinc-deficient ration, no changes were observed involving the hair shaft. Changes of the hair follicle were similar to the epidermal changes in areas near the orifice of the hair follicle. In inflamed areas, the hair follicle was partially obstructed by inflammatory cellular debris. The sebaceous glands were hypertrophied (Figure 8).

Zinc Deficiency and *D. congolensis* Infection

In Experiment 1, the reaction in 2 of the 3 zinc-deficient calves exposed to *D. congolensis* was rather mild and similar, while in the third calf, which was older, the infection was more severe, as indicated by a body temperature of 40.6 C. Control sites (defatted with xylene and swabbed with nutrient broth) had a slight swelling along the scarified marks. By 24 hours (hr) postexposure (PE), edema was present around the scarified areas and mild hyperemia persisted until 72 hr PE. No further gross lesions were observed.

In the 3 zinc-deficient calves, the areas exposed to *D. congolensis* were erythematous by 48 hr PE. In addition, these areas were edematous. Later, small nodules could be detected with the aid of an electric magnifying glass by 96 hr PE. By 144 hr PE, exudation in all sites was observed. When the scabs began to be noticeable, the edematous reaction began to regress. By 193 hr PE, small scabs around the scarifying areas began to unite to form large ones. The scabs were firmly adhered to the hair. Serosanguineous fluid accumulated after the scabs were forcibly removed. The scabs started to detach from the underlying skin by 24 hr PE, and the inflammatory reaction persisted until about 288 hr PE. In the zinc-supplemented, exposed calves, the lesions were less severe.

In Experiment 2, the gross lesions caused by *D. congolensis* in calves fed either the deficient ration or supplemented with zinc were similar to those seen in zinc-deficient calves in Experiment 1, except that the calves supplemented with zinc had more superficial serum exudation in comparison to the calves fed the zinc-deficient ration.

In both experiments, there was evidence of suppuration in the epidermis. The first change was edema. Subsequently, inflammatory cells passed through the epidermis and started to separate the newly formed epidermis from the original one by a dense accumulation of neutrophils. Areas of spongiosis were then observed involving the stratum spinosum beneath the suppurative area. This reaction intensified at about 48 hr PE. At 96 hr PE, the original epidermis was completely replaced by a new epidermis. Simultaneously, the inflammatory reaction persisted around the orifices of the hair follicles. In addition, there was hypercellularity of the new stratum spinosum. The keratohyalin granules were not frequently observed in areas of the stratum granulosum beneath the inflammatory reaction. These areas were replaced by spongiform vacuoles filled with eosinophilic material. Occasionally the spongiform exudation was observed in proximity to the dermal-epidermal junction. No involvement of the dermis was observed, except for an increase in cellularity near the junction. By 144 hr PE, the epidermis continued to have extensive intercellular edema and a progressive exocytosis. Superficial exudate and inflammatory cellular debris between layers of keratin were the major components in the stratum corneum. They subsequently were replaced by crusts of inflammatory cellular debris and layers of parakeratotic stratum corneum (Figure 9). By 192 to 288 hr PE, the underlying cellular debris was firmly attached to the hair shaft. In cases where the crusts

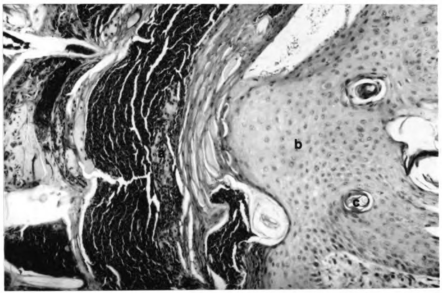

Figure 9. Microscopic appearance of the skin of a calf fed the zinc-deficient ration and exposed to *D. congolensis* (Experiment 2). Crust consists of parakeratotic layer and inflammatory cellular debris (a), acanthotic stratum spinosum (b) and secondary hair follicle (c). Hematoxylin and eosin; 120X.

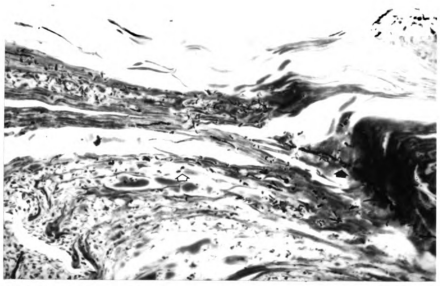

Figure 10. Cocci (clear arrow) and filamentous forms (dark arrow) of *D. congolensis* involving the keratinized layer near the ostium of a hair follicle of a zinc-deficient calf (Experiment 2). Gram's stain; 300X.

had been shed, the stratum corneum was replaced by parakeratotic keratin.

Filaments and cocci of *D. congolensis* were seen in Gram's-stained sections. They were in the stratum corneum mainly around the orifice of the hair follicles by 48 hr PE. When the scarified areas were crusted, the microorganisms were also observed throughout the stratum corneum (Figure 10).

Early in the infection, the histopathologic changes in the dermis were characterized by severe edema and inflammatory cell reaction. The superficial capillary plexus was congested and surrounded by neutrophils at 48 hr PE. By 96 hr PE, there was an intense neutrophilic accumulation beneath the inflamed epidermis. In some calves eosinophils and a few macrophages were seen around the dermal blood vessels at 144 hr PE. Although there were no breaks in the dermo-epidermal junction, there was a marked dermal reaction consisting mainly of neutrophils in areas beneath the epidermal lesions. At that time macrophages and lymphocytes were also present.

The sebaceous glands were only slightly involved in spite of the intense inflammatory reaction. Some of them were partially involved by an inflammatory reaction (Figure 11). However, the reaction was more intense in the apocrine sweat glands, in which the endothelial cells were hypertrophic and the lumen was filled with exudate and neutrophils (Figure 12).

Zinc Deficiency in Chicks

In Experiment 1, the chicks fed the zinc-deficient ration did not develop typical signs of zinc deficiency. This was attributed to zinc contamination from the water and cages. The chicks fed the zinc-deficient ration did retain more of the primary feathers on the lateral

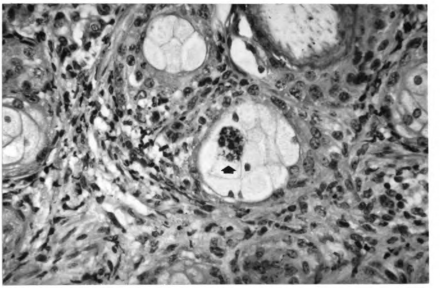

Figure 11. A sebaceous gland from the lateral thorax of a calf fed the zinc-deficient ration and exposed to *D. congolensis* (Experiment 1). Inflammatory cellular reaction in the glandular acinum (arrow). Hematoxylin and eosin; 300X.

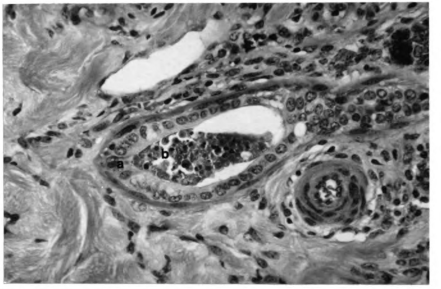

Figure 12. Apocrine sweat gland of the lateral thorax of a calf fed the zinc-deficient ration and exposed to *D. congolensis*. Thickening of the glandular epithelium (a) and inflammatory cells in the lumen (b). Hematoxylin and eosin; 300X.

sides of the body and they had a dry, scaly skin, especially on the ventral parts of the feet, in contrast to the chicks fed the zinc-supplemented ration.

In Experiment 2, clinical signs of zinc deficiency developed quickly in the chicks fed the same type of zinc-deficient ration as in Experiment 1. This was attributed to the use of deionized water and housing the chicks in plastic-coated steel cages.

The following results are based primarily on Experiment 2.

Clinical Signs

Inappetence and lack of growth were the primary signs at the end of the first week of the experiment. By the end of the second week, the feathers of the wings, especially the remiges, had grown slowly and the barbules failed to develop properly. At that time no remarkable growth of secondary feathers was observed in any region of the body. In some chicks, the feathers failed to grow outside of the follicles. The skin of the feet was scaly.

By the end of the third week, the chicks were severely affected by the deficiency. Denuded areas were present around the neck and on the ventrolateral aspects of the body, since the chicks had lost their primary feathers and there was no replacement by secondary feathers. The skin appeared to be dry and scaly on the anterolateral aspects of the body and also on the interdigital areas of the feet. The remiges up to the posterior line of the wings were fragile with continuous atrophy of the barbules and they failed to lace together.

By the end of the fourth week, the zinc-deficient chicks were weak and had an abnormal gait because of the shortening of the tibias, enlargement of the hocks and tender feet. Lack of feathering continued and involved the ventral aspect of the body and the internal aspects

of the thighs. The epidermis was hyperemic in these areas. The ventral aspects of the feet were dry, scaly and easily injured.

By the end of the fifth week, the ventral aspect of the body was completely denuded. The wings were small, with enlarged articulations. The wing feathers were fragile and ruffled. The barbules were completely atrophied, except in those which appeared during the first week of the experiment. The metatarsal foot pads were tender, thick and cracked easily in some chicks. They had difficulty standing.

No clinical signs appeared in subsequent weeks, except for oozing of serosanguineous exudate from inflamed feather follicles. No clinical signs were observed in any chicks fed the ration containing 48.4 ppm zinc nor in the pair-fed chicks.

Laboratory Analysis

Zinc Values. The concentration of zinc in serum and feathers of chicks fed the zinc-supplemented and -deficient rations in Experiment 1 are summarized in Table 2. While clinical signs were not prominent in the chicks in this experiment, the serum zinc values were lower in the chicks fed the zinc-deficient ration. No significant differences were observed between concentration of zinc in feathers.

In Experiment 2, the concentration of serum zinc was significantly different between ration treatments, but the concentration of zinc in the feathers was not significantly different by analysis of variance. The results are summarized in Table 3.

Histopathology

In chicks, the epidermal and dermal layers of the skin are thin compared to mammals of the same size. However, in featherless areas

Table 2. The influence of zinc-supplemented and zinc-deficient rations in the concentration of zinc in serum and feather values in Experiment 1

Treatments	Zinc Values[a]	
	Serum (µg/ml)	Feathers (µg/g)
Zinc-supplemented ration (48.4 ppm zinc)	2.07±0.04 (27)[b]	27.17±0.43 (5)
Zinc-deficient ration (12.0 ppm zinc)	0.98±0.05[c](27)	23.37±0.66[d](5)

[a]The values are expressed in mean ± SE.

[b]Figures in parentheses denote the number of samples.

[c]Different ($p<0.0005$) compared to zinc-supplemented serum values.

[d]NS for feather zinc values.

Table 3. The influence of zinc-supplemented, deficient, pair-fed rations and the infectious bursal disease virus (IBDV) in the concentration of zinc in serum and feathers of young chicks in Experiment 2

Treatments	Zinc Values[a]				
	Serum (µg/ml)			Feathers (µg/gm)[b]	
	Unexposed	Exposed	Means[c]	Unexposed	Exposed
Zinc-supplemented ration (48.4 ppm zinc)	2.10±0.07(6)[d]	2.15±0.31(6)	2.13[x]	103.03±4.69(6)	102.25±2.85(6)
Zinc-deficient ration (12.0 ppm zinc)	0.84±0.09(6)	0.63±0.07(6)	0.74[y]	62.13±2.99(6)	61.72±2.14(6)
Pair-fed	1.76±0.08(6)	1.44±0.09(6)	1.60[z]	98.48±6.81(6)	104.53±2.27(6)

[a]The values are expressed in mean ± SE.

[b]NS for analysis of variance.

[c]The difference of means with different letters (x, y, z) are significantly different by Tukey's test at 1% level.

[d]Figures in parentheses denote the number of samples.

like the tarsus and metatarsus, the epidermis is thicker due to the continuous friction to which these areas are exposed.

Epidermis. The epidermis of the chicks fed the zinc-supplemented ration was not completely definitive in its organization. The stratum basale consisted of cuboidal cells which were arranged in a thick cell layer. When the cells moved to the stratum spinosum, the cells changed from a cuboidal to polyhedral shape. This layer was composed of 1 or 2 cells in thickness (Figure 13). The cells also became vacuolated and flattened to form the stratum corneum. The stratum intermediale was not visible in this area. The metatarsal foot pads had a thicker epidermis in comparison to other areas of the body (Figure 17). The basal layer was lined with columnar cells which were arranged in 1 or 2 rows. In these cells, no mitotic activity was observed. The stratum spinosum consisted of 1 to 3 rows of polyhedral cells, and below this was a thick transitional layer. These cells were highly vacuolated. The vacuolization increased as these cells moved toward the corneal layer. At that point the intracellular substance increased and later the cells became incorporated into the stratum corneum. There was no evidence of a stratum granulosum or stratum lucidum in the metatarsal foot pads.

In the epidermis of zinc-deficient chicks there was a severe hyperkeratosis, which was more extensive around the orifices of the feather follicles (Figure 14). In some chicks, the reaction was mild, with a parakeratotic layer alternating with a layer of normal appearing keratin; but in severe cases the vesicles were filled with exudate within the hyperkeratotic layer. The exudate was PAS-positive. These changes were frequently seen in areas among feather follicles.

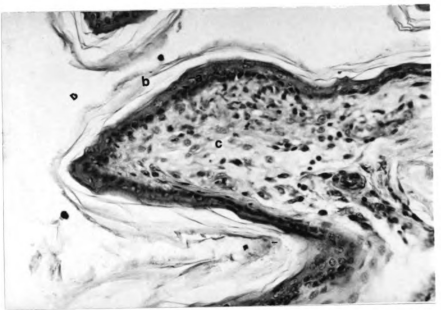

Figure 13. Microscopic appearance of the antero-lateral thoracic skin of a chick fed a zinc-supplemented ration. Stratum germinativum (a), stratum corneum (b), and dermis (c). Hematoxylin and eosin; 120X.

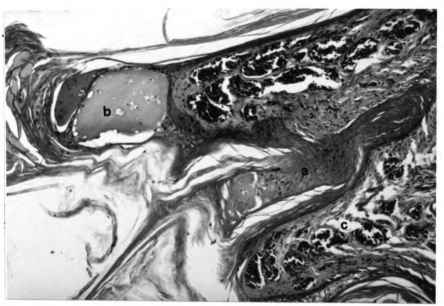

Figure 14. Microscopic appearance of the antero-lateral thoracic skin of a chick fed the zinc-deficient ration. Parakeratosis (a), epidermal vesicle (b), dermal edema and capillary plexus congestion (c). Hematoxylin and eosin; 120X.

The stratum basale was poorly differentiated in zinc-deficient chicks. The cells were arranged in a single layer and they had dark and small nuclei. When the cells had migrated to the stratum spinosum, the keratinocytes had eccentric nuclei and vacuolated cytoplasm. In severe cases, exocytosis was present. It consisted of heterophils migrating from the dermis to the epidermis and accumulating in the spongiform vesicles in the horny layer and contributing to the thickness of the epidermis (Figure 16). The effect of the deficiency was more severe in the epidermis of the metatarsal foot pads. In mild cases, the epidermis was acanthotic and the stratum corneum was relatively thick. In more advanced cases, the normal architecture of the stratum basale was lost and the cells were polyhedral with prominent spherical nuclei. They resembled cells of the stratum spinosum. The cytoplasm of the more superficial layer of the stratum spinosum was pale and hyalinized. Eosinophilic material which resembled abnormal keratinization was usually seen involving the outer layer of the stratum spinosum (Figure 18). Some degree of mitotic activity was observed in the stratum basale. The stratum corneum was also much thicker. Acanthosis was another major component of the dermal lesions and increased in severity when the lesions intensified. This change was frequently observed in areas around feather follicles and in areas of the metatarsal foot pads. The activity of succinic dehydrogenase in the epidermis of the foot pads decreased in a chick fed the zinc-deficient ration in comparison to a chick fed either the zinc-supplemented or pair-fed rations (Figure 19).

Dermis. The dermis of the chicks fed the ration supplemented with zinc consisted of a thin layer of loose connective tissue. The dermis

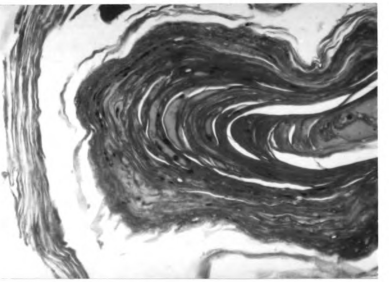

Figure 15. High magnification of the stratum corneum of Figure 14. Parakeratosis of the stratum corneum. Hematoxylin and eosin; 300X.

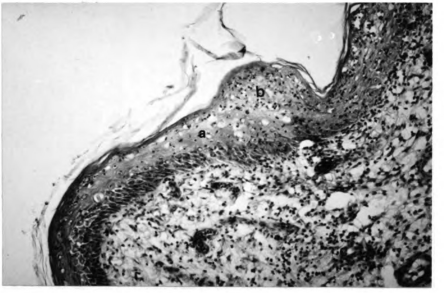

Figure 16. Microscopic appearance of the skin of the wing of a chick fed the zinc-deficient ration. Acanthosis and spongiosis of the stratum spinosum (a), epidermal vesicle filled with heterophils (b), and dermal edema and perivascular lymphocytosis (c). Hematoxylin and eosin; 120X.

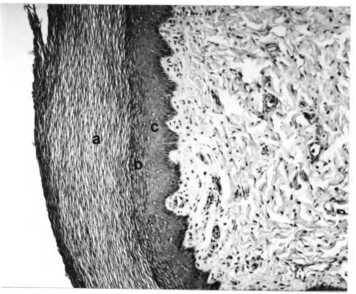

Figure 17. Microscopic appearance of the foot pad of a chick fed zinc-supplemented ration. Stratum corneum (a), stratum intermediale (b), stratum spinosum (c) and stratum basale. Hematoxylin and eosin; 120X.

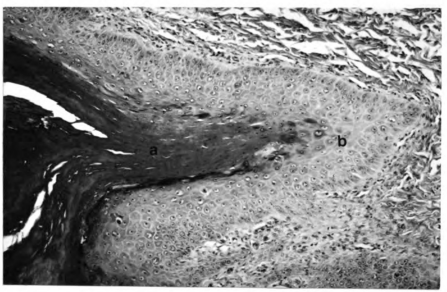

Figure 18. Microscopic appearance of the foot pad of a chick fed the zinc-deficient ration. Hyperkeratosis of the stratum corneum (a), acanthosis and dyskeratosis of the stratum spinosum (b). Hematoxylin and eosin; 120X.

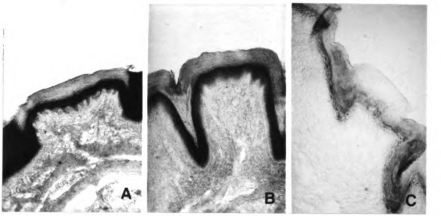

Figure 19. Site of the activity of succinic dehydrogenase (black areas) in the epidermal foot pad of a chick fed a zinc-supplemented ration (a), restricted feed (b), and zinc-deficient ration (c). Nitro BT stain; 32X.

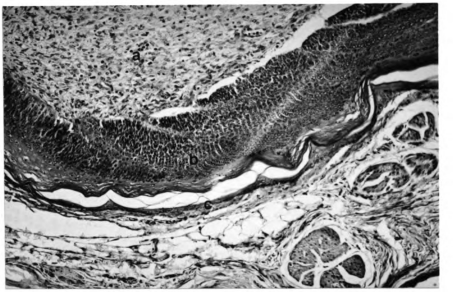

Figure 20. Feather follicle of the wing of a chick fed the zinc-supplemented ration. Dermal papilla (a), epidermal collar of the feather (b), and epidermis of the feather follicle (c). Hematoxylin and eosin; 120X.

had numerous anastomosing superficial capillaries. The dermis of zinc-deficient chicks was edematous, mainly in areas of the wings and in the anterior part of the body. The blood vessels of the superior capillary plexus were congested (Figure 14). These changes occurred in areas where the dermatosis was prominent. The dermal papillae from the metatarsal foot pads were also edematous and the blood vessels had active-appearing endothelial cells and some of them were vacuolated. No significant inflammatory reaction was observed involving the corion, except in areas where the dermatosis was replaced by a severe dermatitis.

Appendages. In the feather follicles of the zinc-supplemented chicks, the epidermis was thin (Figure 20). In the chicks fed the zinc-deficient ration, the uropygial gland was smaller in contrast to the gland in chicks fed the zinc-supplemented ration. The cells appeared to be normal.

In the chicks fed the zinc-deficient ration, the feather follicles had an increase of stratum corneum and a mild thickening of the stratum spinosum. The epidermis was acanthotic and consisted of 3 or more rows of polyhedral cells. Hyperkeratosis was present at the orifice of the feather follicles. As the deficiency progressed, the hyperkeratotic layer extended into the feather follicles. This resulted in complete atrophy of the feather shaft. The stratum corneum was then replaced by a severe parakeratosis (Figures 21 and 22). In areas of the wings, the whole follicle was replaced by a severe granulomatous reaction consisting of heterophils, macrophages, giant cells, bacterial colonies and keratin debris. The surrounding dermis was edematous and heavily infiltrated with heterophils. Lymphocytic perivascular reaction was also observed in the dermal vascular plexus (Figure 23). No lesions were observed in chicks fed the zinc-supplemented ration.

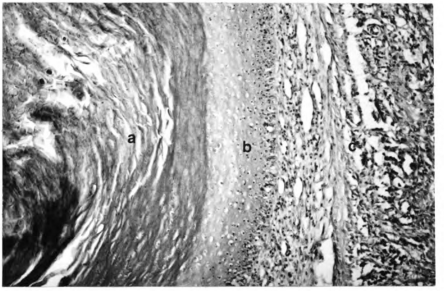

Figure 21. Feather follicle of the wing skin of a chick fed the zinc-deficient ration. Parakeratosis (a) and acanthosis (b) of the epidermis of the feather follicle; dermal edema with heterophil infiltration (c). Hematoxylin and eosin; 120X.

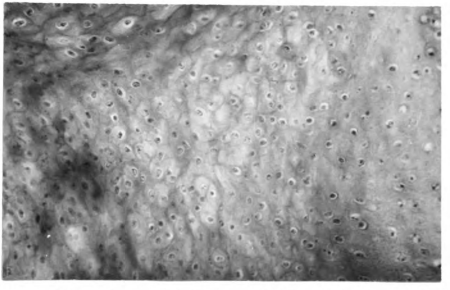

Figure 22. High magnification of Figure 21. Parakeratosis of the stratum corneum of the feather follicle. Hematoxylin and eosin; 300X.

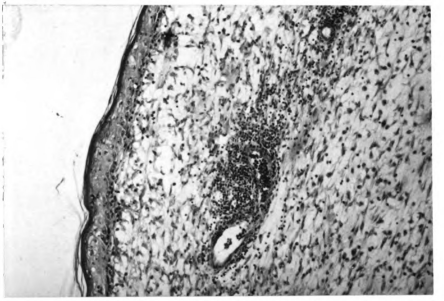

Figure 23. Dermal perivascular lymphocytosis in the skin of the wing of a chick fed the zinc-deficient ration. Hematoxylin and eosin; 120X.

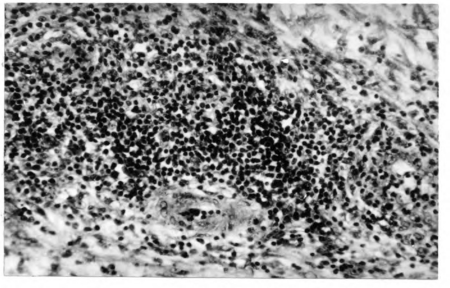

Figure 24. High magnification of Figure 23. Perivascular infiltrate composed of lymphocytes. Hematoxylin and eosin; 300X.

Upper Alimentary System. In the chicks fed the zinc-deficient ration, the tongue was thickened due to an increase of the stratified squamous epithelium, mainly in the posterior region. The keratinocytes were flattened near the surface, were increased in number, and had retained and pyknotic nuclei. The chicks fed zinc-supplemented ration had a thinner squamous epithelial layer and less parakeratosis.

In zinc-supplemented chicks the esophagus had a basal layer lined with columnar type cells arranged in a palisade with sharply demarcated border. In the direction of the esophageal lumen, the stratified squamous epithelium was composed of 6 or 10 rows of cells in which the nuclei were round or oval. Near the lumen the cells were more flattened with pyknotic nuclei and keratinized (Figure 25). In chicks fed the zinc-deficient ration, the basal layer had an increased number of cells, but no apparent mitotic activity was observed. There were 6 to 14 layers of stratified squamous epithelial cells. The cells became more flattened with pyknotic nuclei resembling a parakeratotic condition as they became close to the lumen (Figure 26). Deep crevices appeared to involve the upper portion of the stratified squamous epithelium. The crevices were filled with eosinophilic material containing large numbers of microorganisms.

In the crop of chicks fed zinc-supplemented ration, the general appearance was the same as in the esophagus (Figure 27). In the chicks fed the zinc-deficient ration, the crop was the most affected area of the upper digestive tract (Figures 28 and 29). The basal layer was lined with numerous immature cells piled up in an irregular manner. Near the lumen, the cytoplasm started to be progressively vacuolated and the nuclei were more pyknotic. The lamina propria was edematous and the endothelial cells of the blood vessels were immature. In some

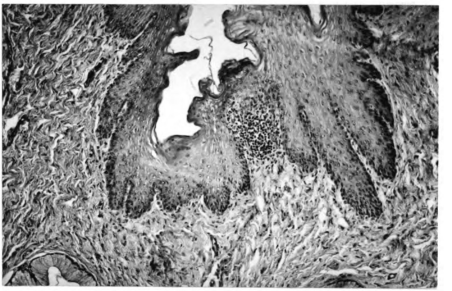

Figure 25. Microscopic appearance of the esophagus of a chick fed the zinc-supplemented ration. Hematoxylin and eosin; 120X.

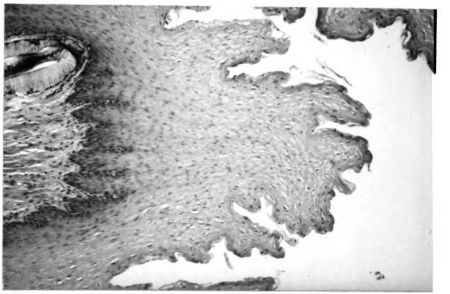

Figure 26. Microscopic appearance of the esophagus of a chick fed the zinc-deficient ration. Acanthosis of the stratified squamous epithelium. Hematoxylin and eosin; 120X.

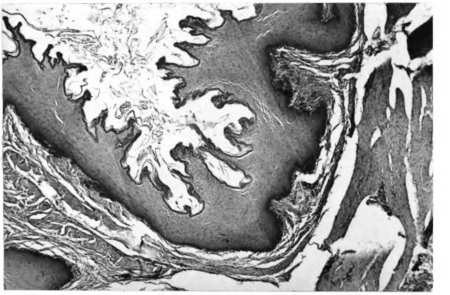

Figure 27. Microscopic appearance of the crop of a chick fed the zinc-supplemented ration. Hematoxylin and eosin; 48X.

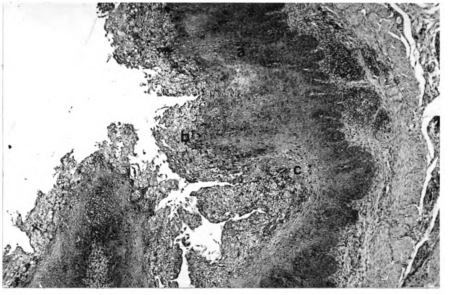

Figure 28. Microscopic appearance of the crop of a chick fed the zinc-deficient ration. Acanthosis of the stratified squamous epithelium (a), vacuolization of the upper portion of the stratified squamous epithelium (b), and heterophils migrating toward the lumen (c). Hematoxylin and eosin; 48X.

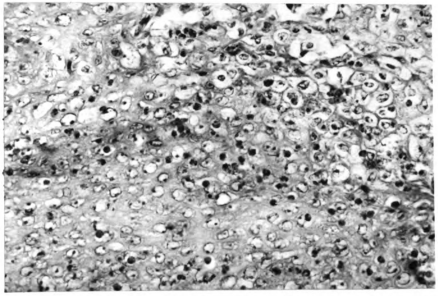

Figure 29. High magnification of Figure 28. Exocytosis in the stratified squamous epithelium. Hematoxylin and eosin; 300X.

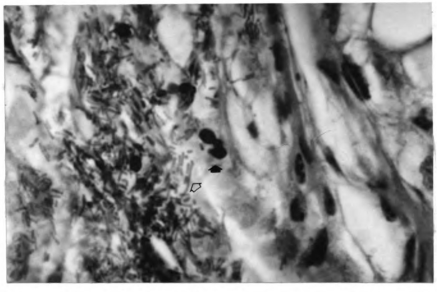

Figure 30. Yeast-like (dark arrow) and bacillus (clear arrow) organisms in the crop of a chick fed the zinc-deficient ration. PAS stain; 1,200X.

severe cases, an inflammatory reaction consisting mainly of heterophils was observed throughout the stratified squamous epithelium as well as in the upper portion of the lamina propria. In some cases the cells near the lumen were embedded with a pinkish material and the mass contained large numbers of yeast-like organisms and bacilli (Figure 30).

Zinc Deficiency and Infection in Pigs

Most of the pigs had a mild diarrhea for 1 to 3 days after the experiment started. This was associated with the change in ration and environment. However, in 1 pig fed the zinc-deficient ration the diarrhea continued during the entire experiment.

Clinical Signs

The skin of the pigs fed the zinc-supplemented ration was characterized by a sparse hair coat, except for hairless areas of the ventral trunk, snout and edges of the lips and hooves. The surface of the snout was smooth and moist. The hair was shiny and flexible.

In the pigs fed the zinc-deficient ration, the first clinical sign at the end of the second week was inappetence and low weight gain (Table 4). By the third week, the hair coat was dry and dull. In some of the pigs, the first area to become involved was the skin of the ears. The ear surface was covered by a spotted erythematous reaction. When exposed to feed and water, it formed a crust. The crust had a moist appearance and was easily removed. Crusts were present in areas that had been involved previously by alopecia, such as the face, posterior aspect of the thighs and perianal region (Figure 31). These areas were constantly subjected to continuous mechanical abrasion. As the zinc deficiency progressed, circumscribed

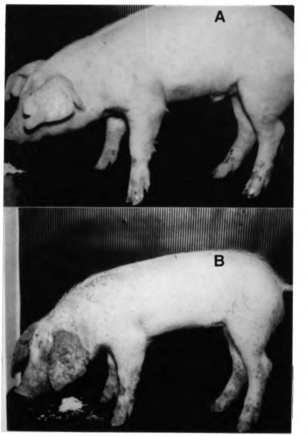

Figure 31. General appearance of a pig fed the zinc-supplemented ration (a) and general appearance of a pig fed the zinc-deficient ration (b).

areas of parakeratosis, 2 to 3 mm in diameter, were observed on the internal surface of the rear legs. At that time, the coronary bands and the dew claws were easily injured on the slotted bottom of the pens.

By the end of the fourth week, the edges and dorsal aspects of the ears were covered by crusted material consisting of a serum exudate and debris. When the crusts were removed, a hyperemic epidermal surface was seen, and it subsequently oozed a serosanguineous fluid. An epidermal reaction covered almost the entire face of zinc-deficient pigs. The skin became scaly and later encrusted by a brownish to dark material which became extensive as the condition progressed. The same condition occurred in areas of the posterior aspect of the thighs and perianal area. At the end of the fifth week, a crusted surface was the prominent lesion of the fetlocks and coronary bands. A linear zone of scaly epidermis was observed on the dorsal aspects of the rump and loin. The chest area of some zinc-deficient pigs had spotted parakeratotic lesions. The sole of the hooves of the zinc-deficient pigs cracked and the keratin fell off easily.

Necropsy

Serous atrophy of fat was observed in the zinc-deficient pigs. It involved the subcutaneous tissues and cardiac coronary sulci. One of the zinc-deficient pigs had a severe edema involving the rear legs and its thymus weighed 3 gm. The thymus of its littermate, fed the zinc-supplemented ration, weighed 77 gm. The tongue of zinc-deficient pigs was thicker in its dorsal aspect, mainly in areas of the filiform papillae, and cracked easily. Deep fissures were present and were filled with greenish to white material. In the cardia of the stomach,

the mucosa was covered by spots of greenish to white material near the esophagic area.

Laboratory Analysis

Serum zinc values, results of hematologic examination, and protein electrophoresis results are summarized in Table 4. Serum zinc values of pigs fed the zinc-deficient ration were significantly different in comparison to the values in the pigs fed the zinc-supplemented ration. Anemia was not present. The total leukocytic count as well as the neutrophilic and lymphocytic values were not affected by dietary treatment.

The zinc-supplemented pigs had higher albumin values and the α-globulin values were increased in the zinc-deficient pigs. The β-globulin values were also decreased in the deficient pigs, while the γ-globulin values were increased.

Histopathology

The skin of the pigs fed the zinc-supplemented ration was remarkably different in various locations of the body. The skin of the snout was especially different. It was flat and had sparse and short vibrissae in its surface. In hairless areas, like the hooves, the stratum corneum was very thick.

Epidermis. The epidermis of the pigs fed the zinc-supplemented ration was thick and had large and small rete pegs. The stratum basale had one thick layer of columnar cells. No significant increase in mitotic activity was observed in this area. The stratum spinosum consisted of polyhedral cells. It was composed of 2 or 5 rows of cells in most areas of the body (Figure 32) but was increased in the hooves and snout. The stratum granulosum was represented by an irregular

Table 4. Mean body weight changes and tissue analyses of pigs fed either zinc-supplemented or zinc-deficient rations[a]

Items	Treatments		P
	Zinc-Supplemented	Zinc-Deficient	
Body weight (kg)	12.11±0.44(36)[b]	10.93±0.72(36)	<0.10
Serum zinc (μg/ml)	0.62±0.05(18)	0.23±0.03(18)	<0.00005
Hemoglobin (g/dl)	12.21±0.53(6)	11.87±0.19(6)	NS
PCV (%)	35.00±1.15	31.00±1.78	<0.05
WBC (no./mm^3)	12080±2690(5)	11170±3262(4)	NS
Neutrophils (no./mm^3)	4696±1052	4427±937	NS
Lymphocytes (no./mm^3)	7196±6595	6685±3210	NS
Total serum protein (g/dl)	5.10±0.19(6)	5.16±0.88(6)	NS
Albumin	2.18±0.11	1.93±0.08	<0.05
α-Globulins	1.40±0.12	1.63±0.02	<0.05
β-Globulins	1.10±0.00	0.81±0.15	<0.025
γ-Globulins	0.43±0.11	0.76±0.18	<0.10
Ration (A/G)	0.74	0.60	

[a]The values are expressed as mean ± SE.

[b]Figures in parentheses denote the number of samples.

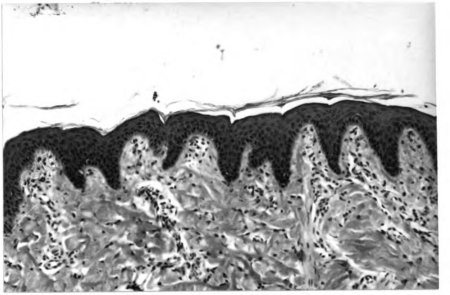

Figure 32. Microscopic appearance of the skin of a pig fed the zinc-supplemented ration. Hematoxylin and eosin; 120X.

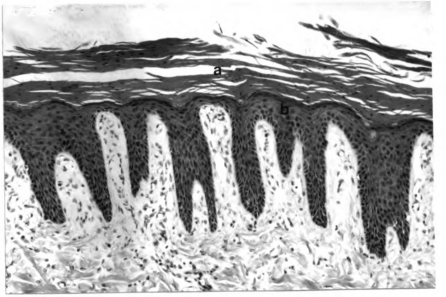

Figure 33. Microscopic appearance of the skin of a pig fed the zinc-deficient ration. Parakeratosis of the stratum corneum (a) and acanthosis of the stratum spinosum (b). Hematoxylin and eosin; 120X.

layer of keratinocytes which was dense around the ostia of the hair follicles. The stratum lucidum was absent. The stratum corneum was composed of a thin layer of flattened and anucleated keratinocytes. The granular layer was differentiated in the area of the snout and the lips. The rete pegs were thinner and deeper in comparison to the other areas of the body.

The epidermis of pigs fed the zinc-deficient ration had a slight acanthosis of the stratum spinosum with bundles of thick stratum corneum. As the reaction became more severe, parakeratosis was observed in areas where the lesions were characterized by brownish spotted areas (Figure 33). In severely affected areas like the ears and face, the crusts consisted of layers of parakeratotic stratum corneum infiltrated by inflammatory cellular debris. In areas affected by the inflammatory reaction, the cells of the stratum basale were separated from each other by intercellular edema. The nuclei of these cells were enlarged and the mitotic activity was increased. Subsequently, the stratum spinosum was partially replaced by a severe suppurative process in the upper portion causing spongiform abscesses, which contributed to the disruption of the epidermis. The stratum granulosum was absent, inasmuch as the keratohyalin granules were not present and the area was replaced by spongiform spaces, mainly beneath the secondary inflammatory processes. Acanthosis was the major component of the most affected area. In the face the rete pegs were elongated and appeared to anastomose with the adjacent ones.

Dermis. The dermis of pigs fed the zinc-supplemented ration consisted of bundles of collagen fibers oriented largely horizontally. There was a substantial papillary area consisting of fine collagen fibers resting between the rete ridges of the snout and corneal areas.

The capillaries formed a moderate capillary plexus inside the dermal papillae.

In zinc-deficient pigs, the dermis was edematous and the endothelial cells of the superficial capillaries were active beneath the areas affected by inflammatory reaction. Inflammatory cells, mainly neutrophils, were observed in areas of the dermis surrounding the lesions in the epidermis and appendages.

Appendages. In zinc-supplemented pigs, the hair follicle was composed of a thin epidermis of the same size and dimension as the epidermis of the skin surface.

The hair coat of the zinc-deficient pigs was replaced by a progressive alopecia in areas affected by the lesions. The hair shaft was usually found in a resting phase. The hair follicle was somewhat acanthotic, mainly in areas near the orifices of the hair follicles, suggesting a possible extension of the acanthosis.

The sebaceous glands were small and associated with the hair follicle. Morphologically, the secretory areas consisted of cells with foamy cytoplasm. The sebaceous glands of zinc-deficient pigs were slightly enlarged and their cells were partially vacuolated.

The apocrine sweat glands of zinc-supplemented pigs were located deep in the dermis. The glandular areas were lined with a monolayer of flattened cuboidal cells and a large lumen. In zinc-deficient pigs, the sweat glands had the same pattern, except for those located in areas affected by secondary microbial infection. They were distended with hypertrophic cuboidal cells. The lumen was filled with inflammatory cells. In severe cases, the glandular area was completely replaced by inflammatory cells, especially neutrophils.

The "eccrine" sweat glands of the snout of zinc-supplemented pigs consisted of coils of glandular structures deep in the dermis. The secretory area of the gland was lined with cuboidal cells. The lumen was not quite visible. In zinc-deficient pigs, areas of focal inflammatory reaction were characterized by neutrophils, lymphocytes and some macrophages.

Upper Digestive System

Tongue. The epithelium of the tongue of zinc-supplemented pigs was characterized by (1) a stratum basale, consisting of a single layer of columnar cells, (2) a stratum spinosum, consisting of 3 or more layers of polyhedral keratinocytes with indistinct cellular borders and large round to oval nuclei, and (3) the stratum corneum, consisting of a thicker layer of keratin, which was more dense in areas of the filliform papillae. In zinc-deficient pigs, the columnar cells of the stratum basale had an irregular arrangement. The stratum spinosum was acanthotic, consisting of 6 to 8 layers of keratinocytes with indistinct and irregular cell borders. The stratum corneum was greatly thickened and in some areas was replaced by parakeratotic stratum corneum. In severe cases, crevices were seen in the stratum corneum with an accumulation of cellular debris, bacterial colonies and filaments and buds typical of *Candida albicans*.

Esophagus. The esophagus of the zinc-supplemented pigs was characterized by a thick stratified squamous epithelium. The basal layer was lined with a monolayer of columnar type cells. The stratum spinosum consisted of 3 to 4 layers of polyhedral keratinocytes. The stratum corneum was characterized by a thin layer of flattened cells with pyknotic nuclei. The lamina propria was composed of a thin layer

of collagen, and beneath this area the esophageal glands were organized in clusters. In zinc-deficient pigs the stratified squamous epithelium was somewhat thickened. The basal layer was arranged in an irregular row of columnar cells. The stratum spinosum consisted of 4 to 6 layers of polyhedral cells which were vacuolated in some areas. The stratum corneum consisted of an irregular parakeratotic layer where accumulation of cellular debris and colonies of microorganisms were present.

Stomach. The area most affected by zinc deficiency was the cardia. Acanthosis of the stratified squamous epithelium and parakeratotic stratum corneum were present. Large numbers of *Candida albicans* were observed.

Zinc Deficiency and *S. hycus* Infection

No clinical signs of "greasy pig disease" were observed in the zinc-supplemented or in the zinc-deficient pigs exposed to *S. hycus*. Histologically, wounds of zinc-deficient pigs exposed to the pathogen were characterized by a severe suppurative process. The upper portion of the epidermis was replaced by spongiform abscesses. In these areas the hair follicles were filled with neutrophils and fibrin strands. Gram-positive bacterial colonies were observed in association with the hair shaft. The lumens of the apocrine sweat glands were filled with inflammatory cells, mainly neutrophils. Gram-positive colonies of cocci and saprophytic fungi, such as *Candida albicans* and *Alternaria* sp., were in close association with the injured epidermis before and after exposure to *S. hycus*. The same lesions were observed in the hair follicles.

DISCUSSION

Skin changes were the important clinical signs and lesions of zinc deficiency in calves, chicks and pigs. Lesions in the stratum corneum were confirmed to be an initial sign of zinc deficiency in the calf and pig. Zinc-deficient animals were susceptible to skin infection whether of experimental or natural origin.

Comparative Dermatopathology

The integument of the zinc-supplemented calves, chicks and pigs was similar in histological features to that described for the skin of the calf (Sinha, 1964), chick (Lucas and Stettenhein, 1972) and pig (Marcarian and Calhoun, 1966). Although the normal skin of the 3 species examined in this research had similar anatomical and histological features, there were differences. In all 3 species the epidermis was composed of stratified squamous epithelium in 3 distinct layers: the stratum basale, the stratum spinosum, and the stratum corneum. In addition, the stratum granulosum was present in the calf and pig and the stratum lucidum was common in the calf. The stratum granulosum and the stratum lucidum were represented in the chick by the stratum intermediale. The dermis, which was essentially loose connective tissue, was thicker in the pig and poorly vascularized in comparison to the dermis of the calf and chick. Sebaceous glands were numerous in the calf, reduced in the pig, and represented by the uropygial gland in the chick. Over the body surface of the pig and calf, apocrine

sweat glands were numerous but were absent in the chick. The "eccrine" sweat glands were numerous in some specialized areas like the muzzle of the calf and the snout of the pig. However, they were absent in the chick.

Involvement of the skin was characteristic of zinc deficiency in animals, especially the calf (Miller and Miller, 1962), chick (O'Dell et al., 1958), and pig (Voelker, 1970). In this research the early lesions of zinc deficiency in the calf and pig were characterized by epidermal changes and dermal edema of the superficial capillaries. These lesions were similar to the early lesions of psoriatic patients (Ragaz and Ackerman, 1979). Most likely the early lesions in chicks were also similar.

Parakeratosis was a characteristic lesion of zinc deficiency in the calf (Miller and Miller, 1962), pig (Tucker and Salmon, 1955) and rat (Follis et al., 1941). It was also mentioned as a characteristic lesion in psoriasis in man (Anderson et al., 1967). However, parakeratosis was not the main feature found in the skin of the deficient chicks (O'Dell et al., 1958). In this study parakeratosis in the chick as well as in the calf and pig was associated with areas where the keratinocyte turnover was increased due to zinc deficiency and constant mechanical trauma. Zinc-dependent enzymes are required in the normal growth, differentiation and shedding of the keratinocyte. The severe hyperkeratosis of the foot pads of the chicks consisted of early maturation and severe degeneration of the keratinocyte of the stratum spinosum.

The sebaceous glands were enlarged in the calf and slightly enlarged and vacuolated in the pig. The "eccrine" sweat glands were more inflamed in the pig than in the calf. The apocrine

sweat glands were inflamed in zinc-deficient calves and pigs.

In zinc deficiency, the integumentary tissue of all 3 species was more susceptible to microbial infection than in zinc-supplemented animals.

Zinc Deficiency and Skin Infection in Calves

The skin of the calves fed the basal ration supplemented with zinc had the same morphological features as the healthy Holstein calf (Sinha, 1964).

The clinical signs and lesions of zinc deficiency did not follow the same sequence in each calf in either experiment, inasmuch as in some of the calves fed the deficient ration typical signs of a deficiency did not develop. Similar difficulties were reported by Ott et al. (1965). In contrast, Mills et al. (1967) mentioned that calves as well as lambs, when fed a zinc-deficient ration, had rather uniform clinical signs. These differences in results in calves may be due to the role of microbial factors in the rumen in making zinc available and also may be related to zinc contamination from the environment.

In the calves, the epidermal lesions seemed to be most prominent in areas subjected to continuous friction. This was also observed in zinc-deficient calves by Miller and Miller (1962). In areas where alopecia was prominent, the stratum corneum was thicker and in some areas there were mounds of parakeratotic stratum corneum. This lesion was described as one of the early changes in the skin of psoriatic human patients (Ragaz and Ackman, 1979). In the severely affected areas of calves, the crusted material consisted of thick layers of parakeratotic stratum corneum and inflammatory cellular debris. The stratum granulosum beneath the parakeratotic layer was progressively replaced by spongiosis, which resulted from early intra- and intercellular

edema. No significant changes in this layer were observed around the orifice of the hair follicle. Spongiosis was also reported in human patients suffering from acrodermatitis enteropathica (Wells and Winkelmann, 1961) and psoriasis (Ragaz and Ackerman, 1979). No reference to spongiosis was made by Ott et al. (1965), Miller and Miller (1962) or Mills et al. (1967) in zinc-deficient calves.

The dermis beneath the areas affected by spongiosis was edematous and the blood vessels of the superior plexus were prominent and were lined with enlarged endothelial cells. This condition was also mentioned in zinc-deficient pigs by Voelker (1970) and it was suggested to be an early lesion in psoriatic skin in man by Pinkus and Mehregan (1966). Recently, Braverman (1977), using an electron microscope, confirmed that this lesion was related to changes in the capillary wall in patients with psoriasis. No changes were observed in the apocrine sweat gland of these zinc-deficient calves, as has been reported for zinc-deficient pigs (Voelker, 1970). The sebaceous glands were somewhat enlarged in the zinc-deficient calf, and the same observation was made in the rat by Follis et al. (1941).

The response of calves to exposure to *D. congolensis* was different in Experiments 1 and 2. In Experiment 1, there was a longer period of time for the appearance of lesions. The lesions were the same in zinc-supplemented (150 mg 3 times a week) and unsupplemented calves in Experiment 1.

In Experiment 2, lesions due to *D. congolensis* infection were milder and serum zinc values were higher in the calves supplemented with 200 mg zinc daily. Serum zinc values were not decreased following exposure to *D. congolensis* in either experiment. These results are in contrast to the temporary decrease in serum zinc values in response to

viral hepatitis (Henkins and Smith, 1972), live vaccine of *Francisella tularensis* and to endotoxin of *E. coli* (Pekarek and Evans, 1975). Zinc supplementation (150 mg 3 times a week) did not appear to influence serum zinc values in Experiment 1. The values in both groups were low, but not as low as the value of 0.4 µg/ml serum, as suggested by Mills et al. (1967) for deficient calves. In Experiment 2, the zinc-supplemented (200 mg zinc daily) calves had significantly higher serum values, yet the values for the unsupplemented calves were about the same as the unsupplemented calves in Experiment 1. An important reason for the difference in response to zinc supplementation between Experiments 1 and 2 would be the deleterious effect the egg-white protein had on the calves in Experiment 1. Although the calves in Experiment 1 appeared to recover from the effect of the egg-white protein after it was discontinued, they tended to remain unthrifty and more susceptible to respiratory infections. The inappetence and enteric disturbances associated with feeding the egg-white initially in Experiment 1 could have induced a general malnutrition that modified the results of zinc supplementation and exposure to *D. congolensis* infection, as suggested by Whitehair and Miller (1974) and Scrimshaw et al. (1959). While the egg-white appeared to complicate the results in Experiment 1, the research did allow the development of techniques and procedures for Experiment 2 and provided information of value under field conditions of malnutrition due to diarrhea. The experiment also provided information on the deleterious effect of egg-white protein as a feed for calves. It can be used as a protein low in zinc for other species, especially laboratory animals, but it cannot be tolerated by calves.

The histopathological changes associated with *D. congolensis* infection consisted of acanthosis of the stratum spinosum and parakeratosis

of the stratum corneum. The lesions were similar to those described in naturally occurring cases of *D. congolensis* in cattle (Kelly et al., 1964; Pier et al., 1963; Shotts et al., 1969). The ostia of the hair follicles were invaded by cocci and filamentous forms of *D. congolensis*. Unlike experiments with sheep and rabbits, in which the microorganisms were observed in large numbers (Roberts, 1965), only a few microorganisms were observed in the keratinous layer around the orifice of the hair follicle in this research, and this was similar to the previous observation of Oduye (1975) in the skin of Nigerian cattle. Although most of the filamentous organisms were observed in the ostia of the hair follicle, they were also seen in keratinized layers of the interfollicular spaces and in the small and large scabs. This was also observed by Oduye (1976). Kelly et al. (1964) in Kansas did not observe involvement of the hair follicle in cattle infected naturally. The observation in this research that *D. congolensis* involved the sebaceous glands was also described in naturally infected Zebu cattle in Nigeria by Amariki (1976).

The hematologic values observed in this study were in the normal range, as suggested by Krehbiel (1976),[1] except for the decreased lymphocytic values observed in the calf fed the zinc-supplemented ration that was exposed to *D. congolensis* infection.

The serum albumin values appeared to be reduced in all the calves of Experiment 1 and in the calves fed the zinc-deficient ration exposed or unexposed to *D. congolensis* in Experiment 2. These results were similar to those reported by Pekarek and Powanda (1976) when zinc-deficient rats were exposed or unexposed to *F. tularensis*.

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Zinc Deficiency in Chicks

The skin of zinc-supplemented chicks was similar in morphology to the skin of the normal White Leghorn chick (Lucas and Stettenhein, 1972).

The use of plastic-coated steel cages and deionized water in Experiment 2 to enhance the development of zinc deficiency in chicks emphasizes the importance of zinc contamination due to factors other than the ration. This was reported previously by Edwards et al. (1958).

The clinical signs of zinc deficiency in chicks included a lack of proper feathering and retention of the primary feathers. These findings were also reported in chicks by Roberson and Schaible (1958) and O'Dell et al. (1958). The same results were mentioned for turkeys (Kratzer et al., 1958), pheasants (Scott et al., 1959) and Japanese quail (Spivey-Fox and Harrison, 1964). Values for zinc in serum and feathers have not been reported previously in chicks. Mills et al. (1967) mentioned that serum zinc values were an important measure of zinc nutrition.

The major lesions were observed in skin areas easily exposed to continuous abrasions. This resulted in scaly lesions with partial retention of secondary feathers in the ventral pectoral regions of the body exposed to continuous friction against the feeder. The scaly and thick foot pads were associated with the zinc-deficient ration and exacerbated by the continuous contact over the slotted bottom of the cages. When the chicks developed abnormal gait due to enlargement and distorted hock joints, they tended to maintain equilibrium by using the wings as support. As a result, the extremities of the wings became enlarged and the feathers broke easily. There was oozing of sero-sanguineous fluid by the feather follicle. This finding was similar to the lesions reported in zinc-deficient calves by Miller et al. (1965).

Parakeratosis has been considered as the major lesion of zinc deficiency in calves, pigs and rats. It is also the lesion associated with psoriasis in man, according to Anderson et al. (1967). However, parakeratosis was not described for zinc deficiency in chicks (O'Dell et al., 1958) or ducks (Wight and Dewar, 1976). In chicks, hyperkeratosis was considered as the predominant lesion. In this research, hyperkeratosis alone was not considered as the most important lesion since other lesions, such as acanthosis, parakeratosis and spongiosis, appeared as the deficiency progressed. On the ventral pectoral aspect of the body, the lesions were mainly spongiosis. On the other hand, mounds of parakeratotic stratum corneum were observed in areas of spongiosis. The mounds of parakeratotic tissue were similar to those in zinc-deficient calves in this research and in lesions of psoriasis in human patients (Ragaz and Ackerman, 1979). The epidermis of the foot pads was thicker, having an aspect of severe acanthosis with areas of early maturation of keratinocytes characterized by dyskeratosis. An observation similar to this was reported in the duck by Wight and Dewar (1976). The decreased amount of succinic dehydrogenase in the foot pads of zinc-deficient chicks was similar to that reported in the skin of rats by Im et al. (1975). The skin of the wing was the most affected area at the termination of the deficiency. The feather follicle was replaced by a severe granulomatous reaction consisting of macrophages, giant cells and keratin debris. This observation was not mentioned in previous work (O'Dell et al., 1958). The epidermis of the feather follicle had a thicker parakeratotic stratum corneum in areas where the feather shaft was atrophied. This was mentioned by Voelker (1970) in zinc-deficient pigs but was not observed in chicks by O'Dell et al. (1958) nor in ducks by Wight and Dewar (1976).

The dermal lesions of edema, heterophil infiltration and perivascular lymphocytosis in zinc-deficient chicks have not been reported previously.

The esophageal lesions consisting of acanthosis of the stratified squamous epithelium in this research were not mentioned previously in the chick but were reported in the duck (Wight and Dewar, 1976) and rat (Whitenack, 1968). In the present research, the crop was the most affected part of the upper digestive tract. The lesions consisted of thickening of the stratified squamous epithelium with a progressive cellular vacuolization towards the surface. These findings were not reported in zinc-deficient chicks (O'Dell et al., 1958) nor in zinc-deficient ducks (Wight and Dewar, 1976). Infections had been reported in zinc-deficient pigs (Whitenack et al., 1978) and were observed in the chicks in this research.

Zinc Deficiency and Skin Infection in Pigs

In this research the clinical signs of zinc deficiency in pigs were characterized by inappetence, reduced growth rate and parakeratotic lesions. The same results were observed in the baby pig (Miller et al., 1968).

As in the calf and chick, the major lesions of zinc deficiency were located in areas exposed to continuous abrasion. These were the scaly lesions on the surface of the face, dorsal aspect of the ears, fetlocks and thighs. Voelker (1970) observed these early lesions on the ventral aspect of the body and on the perianal area. The findings were probably modified by the type of cage used in each experiment.

In areas affected by the early lesions of zinc deficiency, the stratum corneum was hyperkeratotic. This finding was in contrast with

the parakeratosis in the early lesions in zinc-deficient calves and reported in the skin of psoriatic patients (Ragaz and Ackerman, 1979). In the epidermis of zinc-deficient pigs, the stratum granulosum was absent, except in areas around the hair follicles where the keratohyalin granules were present. This was mentioned previously for the calves and was a common factor in psoriatic patients (Ragaz and Ackerman, 1979; Milne, 1972).

Acanthosis of the stratum spinosum was a prominent lesion in zinc-deficient pigs (Voelker, 1970), in calves (Miller and Miller, 1962) and also in patients with epidermal diseases (Well and Winkelmann, 1961; Ragaz and Ackerman, 1979). In this research, acanthosis was associated with early lesions of zinc deficiency, but later the epidermis over the dermal papillae was thin and the rete pegs were thin and elongated. This is typical in severe lesions of psoriasis (Milne, 1972).

The enlarged and vacuolated sebaceous glands of zinc-deficient pigs were similar to the zinc-deficient calves in this research and in the rat (Follis et al., 1971).

The changes in the apocrine sweat glands in the skin of zinc-deficient pigs were similar to those seen in calves exposed to *D. congolensis* in this research.

The focal inflammatory reaction observed in the "eccrine" sweat glands of the snout of the zinc-deficient pig has not been reported previously.

In spite of the negative clinical signs in pigs exposed to *S. hycus*, the hair follicles of the unexposed zinc-deficient pigs were affected by a severe folliculitis. This consisted of inflammatory cells and colonies of gram-positive bacteria and *Candida albicans*.

This may suggest that the pigs had a previous contact with microorganisms and this made them resistant to the experimental infection.

SUMMARY

Five experiments were conducted using 20 calves, 148 chicks and 12 pigs to determine the morphologic and pathologic features of the integumentary tissue, especially the skin, during health and zinc deficiency. In addition, the comparative dermatopathology of zinc deficiency in these 3 species was evaluated. The interrelationships between zinc deficiency and an experimental epidermal infection were also determined in calves exposed to *Dermatophilus congolensis* and pigs exposed to *Staphylococcus hycus*. Zinc deficiency was characterized by serum zinc values, hematological determinations, protein electrophoresis, clinical signs, and gross and microscopic lesions. The same techniques were used in all 3 species.

In calves the clinical signs of zinc deficiency were inappetence, hypersalivation, tender fetlocks, alopecia and crusted skin lesions involving especially the legs. In chicks the clinical signs of zinc deficiency were inappetence, reduced growth rate, poor and abnormal feathering, abnormal gait and inability to stand, scaly dry skin and thickening of foot pads. In pigs the clinical signs were inappetence, reduced growth rate and crusted, erythematous epidermal lesions on the face, ears, and feet. Serum zinc values were decreased in all 3 species and the values correlated with the manifestations of zinc deficiency. A dermal biopsy instrument was most useful in following the microscopic skin changes during the deficiency in calves and pigs.

In calves the microscopic lesions consisted of a parakeratotic stratum corneum, acanthosis and elongated rete pegs that anastomosed with each other. In severe cases the stratum granulosum was replaced by spongiosis except in areas around the hair follicles. In chicks, microscopic lesions included hyperkeratosis and acanthosis with dyskeratosis in the foot pads. The epidermis of the feather follicles on the wings was parakeratotic and acanthotic. In pigs the epidermal lesions were parakeratosis and acanthosis with elongation and anastomosis of rete pegs. In all 3 species the epidermal lesions were similar and involved primarily the keratinocyte. In the chick, however, the hyperkeratosis was more prominent than in the calf and pig. Parakeratosis was present in areas of the wing. Abrasions and presence of microorganisms were factors that modified the nature of the lesions.

Skin lesions of *D. congolensis* infection were more severe in zinc-deficient than in zinc-supplemented calves and consisted of a parakeratotic stratum corneum, acanthosis, spongiform abscesses and hidradenitis. Typical clinical signs of *S. hycus* infection were not produced in pigs, but in the zinc-deficient pigs the hair follicles had a suppurative folliculitis related to gram-positive microorganisms.

Zinc deficiency has a similar effect on the integumentary tissue of calves, chicks and pigs. The tissue changes would increase susceptibility to microbial, parasitic, or chemical injury. Supplemental zinc in livestock rations would most likely improve resistance to skin diseases.

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APPENDIX

Table A1. Hematologic results of calves at the end of phase A, and after exposure with D. congolensis in Experiment 1[a]

Items	Phase A		2 Weeks After Exposure	
	+Zn	-Zn	+Zn	-Zn
Hemoglobin (g/dl)	9.30± 0.66(8)	9.20± 6.04(16)	8.20± 0.51(6)	9.00± 0.62(18)
PCV (%)	26.60± 1.20	27.00± 1.35	24.50± 0.70	24.90± 0.51
WBC (no./mm^3)	4163.00±379.00	5222.50±298.50	2638.00±135.10	4522.22±256.00
Neutrophils (no./mm^3)	886.00±341.70	918.26±349.10	717.20±105.00	1699.85± 14.50
Lymphocytes (no./mm^3)	3179.00±280.70	3896.00±253.90	1647.60±177.60	2467.57±394.50

[a]The values are expressed in mean ± SE.

[b]Figures in parentheses denote the number of samples.

Table A2. Hematologic results of calves at the end of phase A, and after exposure with D. congolensis in Experiment 2[a]

Items	Phase A		1 Week After Exposure	
	+Zn	-Zn	+Zn	-Zn
Hemoglobin (g/dl)	10.60± 0.42(8)[b]	10.58± 0.26(12)	10.09± 0.10(6)	10.71± 0.35(9)
PCV (%)	31.25± 0.95	32.16± 0.65	29.00± 0.40	31.00± 0.90
WBC (no./mm^3)	10881.00±1299.38	11564.00±2050.46	7644.00±741.01	9063.00±1516.00
Neutrophils (no./mm^3)	4169.00±1667.10	4621.00±1028.11	1696.00±345.47	4197.00± 889.75
Lymphocytes (no./mm^3)	6438.00± 413.73	7121.00±1072.26	5622.00±564.89	5230.00± 776.36

[a]The values are expressed in mean ± SE.

[b]Figures in parentheses denote the number of samples.

Table A3. Protein electrophoresis values in serum at phase A, and after exposure to D. congolensis in Experiment 1[a]

Items (g/dl)	Values			
	Phase A		2 Weeks After Exposure to Infection	
	+Zn	-Zn	+Zn	-Zn
Total serum protein	5.40±0.13(12)[b]	5.50±0.10(28)	5.03±0.06(7)	5.18±0.04(24)
Albumin	2.66±0.29	2.59±0.04	2.60±0.40	2.42±0.05
α-Globulins	0.93±0.02	0.94±0.02	0.99±0.03	0.97±0.03
β-Globulins	0.66±0.07	0.66±0.03	0.54±0.20	0.71±0.03
γ-Globulins	1.13±0.08	1.29±0.05	0.85±0.21	1.09±1.10
Ratio (A/G)	1.01	0.90	1.09	0.90

[a]The values are expressed in mean ± SE.

[b]Figures in parentheses denote the number of samples.

Table A4. Protein electrophoresis values in serum collected at the end of phase A, and after exposure to D. congolensis in Experiment 2[a]

Items (g/dl)	Phase A		1 Week After Exposure	
	+Zn	-Zn	+Zn	-Zn
Total serum protein	6.30±1.34(13)[b]	6.03±0.20(25)	6.00±0.18(6)	5.92±0.09(9)
Albumin	3.05±0.26	2.73±0.10	3.13±0.08	2.71±0.16
α-Globulins	1.13±0.08	0.97±0.04	1.02±0.05	1.00±0.08
β-Globulins	0.88±0.06	0.82±0.06	0.75±0.06	0.76±0.07
γ-Globulins	1.28±0.22	1.37±0.11	1.12±0.16	1.49±0.07
Ratio (A/G)	0.98	0.87	1.14	0.84

[a]The values are expressed in mean ± SE.

[b]Figures in parentheses denote the number of samples.

VITA

VITA

The author was born in Tràs-os-Montes, Portugal, on February 12, 1947. He received his primary education in Rio de Janeiro, Rio de Janeiro, and his secondary education in Sao Jose dos Pinhais, Parana. He graduated from the School of Veterinary Medicine, Federal Rural University of Rio de Janeiro, in 1972. He was appointed as an instructor in 1973 and is currently a faculty member in the Department of Veterinary Parasitology, Federal Rural University of Rio de Janeiro, 23.460 -Seropèdica, Rio de Janeiro, Brazil.

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