

AN ABSTRACT OF THE THESIS OF

Madeline Ann Rae, DVM for the degree of Master of Science in  
Veterinary Science presented on December 11, 1997.

Title: Degenerative Myopathy in Ratites

Abstract approved: \_\_\_\_\_

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Although there is abundant scientific information regarding vitamin E and selenium deficiency disease in domestic poultry, there is only scant information on exotic species, such as ratites. Several necropsy cases involving ostriches, emus and rheas were presented to the Oregon State University Veterinary Diagnostic Laboratory with gross and/or histologic lesions of degenerative myopathy. Investigation of these cases led to determination of tissue levels of vitamin E and selenium and to determination of the dietary levels of these same substances. A small group of emus with a post-mortem diagnosis of degenerative myopathy was supplemented with dietary vitamin E with excellent clinical results. This success prompted a survey of vitamin E and selenium levels in blood and feed from clinically normal ratites. This information was then used to make recommendations for dietary levels of vitamin E and selenium in ratite formulated diets. Since overzealous supplementation can result in selenium toxicosis in birds, a safe, but effective level of dietary selenium and vitamin E is necessary for optimal propagation of the various ratites species.

Degenerative Myopathy in Ratites

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of  
the requirements for the  
degree of

Master of Science

Completed December 11, 1997

Commencement June 1998

Master of Science thesis of Madeline Ann Rae, DVM presented on December 11, 1997

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## ACKNOWLEDGMENTS

I would like to sincerely thank Dr. Stanley P. Snyder for accepting me as a graduate student and for not giving up on me through all these years. I extend my gratitude to Karen Walker and Dr. Lori Walker for their assistance and advice in the laboratory. I thank Dr. Deborah Barber-Axthelm for encouraging me in pursuit of this thesis. Many thanks are offered to the host of practicing veterinarians, including, but not limited to, Dr. Amy Raines, Dr. James Stewart, Dr. Thomas Tully, Jr. and Dr. Joseph Snyder, who submitted cases and samples for this research. Sincere thanks are extended to Bryan Wright, who helped me with the statistical analysis on such short notice. Finally, a big sigh of relief for my husband, Max Rae, and my two sons, Matt and Mark, who have lived under the shadow of “the thesis” for so many years.

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# DEGENERATIVE MYOPATHY IN RATITES

## INTRODUCTION

Ratite is the name given to a small group of flightless birds that share a unique shape to their sternums. The term, ratite, comes from the Latin word, *ratitus*, meaning raft. Ratites have a flattened, raft-like sternum that lacks the normally prominent keel seen in other avian species. The term ratite is not a true scientific classification, but does describe these flightless birds which are considered to have evolved from unrelated groups of flighted birds that adapted to a highly specialized terrestrial lifestyle.

The members of the ratite group include the ostriches, the emu, the rheas, the cassowaries, the kiwis and the tinamous.

The ostrich is native to the continent of Africa and is the largest living bird, with adult males standing over 8 feet in height and weighing as much as 350 pounds. The ostrich is in the order Struthioniformes, family Struthionidae and the nominate race is *Struthio camelus*, because its odd-shaped, two-toed feet, domed-shaped profile and desert living conditions are reminiscent of the camel. Four subspecies are currently recognized, with a fifth subspecies, *Struthio camelus syriacus*, the Syrian ostrich from the Arabian peninsula, becoming extinct in 1941. The North African ostrich, *Struthio camelus camelus*, ranges from Morocco to Ethiopia and Uganda. The males of this race have a red pigmentation to their necks and legs and a featherless crown on their heads. The East African or Masai ostrich, *Struthio*

*camelus massaicus*, is found in Kenya and Tanzania. The males of this race have red necks and legs, but feathered crowns. The Somali ostrich, *Struthio camelus molybdophanes*, is found in Somalia and Ethiopia. The males of this race have blue necks and legs and bald crowns. The South African ostrich, *Struthio camelus australis*, ranges throughout southern Africa. The South African males have gray necks and the head is completely feathered. The females of all the subspecies are indistinguishable.<sup>1</sup>

The natural diet of ostriches is varied and includes plants, seeds, fruits, a variety of insects, and small reptiles and rodents. In captivity, they are fed a pelleted ration, composed primarily of alfalfa, supplemented by grazing.

Although wild ostriches can still be found in Africa, selective breeding of ostriches has been practiced on farms in South Africa since the 1870's, primarily for the ostrich feather market. This selective breeding resulted in a smaller, calmer hybrid ostrich with premium feather quality, known either as a "domestic" ostrich or a "South African black" ostrich. The South African black ostrich is a combination of the South African ostrich (*S.c. australis*), the Masai ostrich (*S.c. massaicus*) and the now-extinct Syrian ostrich (*S.c. syriacus*). Here in the United States, the various races of ostriches are referred to as South African blacks, red-necks (Masai and North African subspecies), and blue-necks (Somali and South African subspecies). However, even the red-necks and blue-necks have been hybridized, due to the inability to accurately identify the subspecies of the females. The lack of estrogen in

the adult male ostrich results in distinct black and white plumage, while the plumage of females and juveniles is a dull brown.<sup>1</sup>

In the 1870's, ostriches were primarily raised for their feathers, which were used in the fashion industry on ladies' hats. Ostrich feathers are still utilized today in the making of theatrical costumes and feather dusters. Ostrich skins are highly prized as an exotic leather primarily for its suppleness and for the unique texture produced by the feather follicles. Ostrich meat is also a valuable commodity. This lean red meat has the nutritive and cholesterol value of poultry, but has the texture and flavor of beef.

In the 1980's, approximately 100,000 ostriches were being raised on 400 farms in South Africa, as part of a multi-crop rotation system. In the United States, over 10,000 ostriches are currently estimated to be in production.<sup>2</sup> A ban on importation of ostriches from Africa resulted in high prices being demanded for breeding ostriches and their eggs in the United States and Canada. As production increased in the United States, through growing knowledge about management techniques, nutrition and artificial incubation, prices have dropped and there are attempts to create and maintain markets for the meat and skins around the world.

The emu, *Dromaius novaehollandiae*, is native to Australia and is classified in the order Casuariiformes, family Dromiceidae. It is the world's second-largest living bird, standing 5 to 6 feet in height and weighing up to 120 pounds. The natural diet of the emu includes vegetation and insects. The cassowary, closely related to the emu, is a large, heavy, powerful ratite that is native to northern Australia, New

Guinea and adjacent islands. It is also classified in the order Casuariiformes, but in the family Casuariidae. There are three species of the cassowary: the double-wattled cassowary (*Casuarius casuarius bicarunculatus*), the single-wattled cassowary (*Casuarius casuarius unappendiaulatus*) and the southern cassowary (*Casuarius casuarius johnsonii*). The cassowary has a large, horny casque on the top of its head, dark hairlike feathers and a knife-like claw on their medial digit. This weapon and their natural aggressiveness makes them dangerous to humans. They stand 4 to 5 feet tall and may weigh up to 160 pounds; their diet is primarily composed of fruits.<sup>3</sup>

The South American pampas are the home of the rheas, members of the order Rheiformes. The common or grey rhea, *Rhea americana*, is the most commonly kept rhea species in the United States. Rheas stand only 5 feet tall and weigh 50 to 80 pounds, but they are the largest birds in the western hemisphere. The Darwin's rhea, *Pterocnemia pennata*, is smaller and lives in the eastern foothills of the Andes. Several subspecies of both rheas are classified by their geographic location. Their diet is mostly native vegetation, insects and small mammals.<sup>2,3</sup>

The kiwi of New Zealand is a unique bird even among ratites. The kiwi is a member of the order Apterygiformes. There are four species in the *Apteryx* genus: the common brown or South Island kiwi (*Apteryx australis*), the North Island kiwi (*Apteryx inantelii*), the great spotted kiwi or roa (*Apteryx haasti*) and the little spotted kiwi (*Apteryx owenii*). Kiwis are the chicken-sized, flightless birds with hairlike feathers, weighing between 2 and 8 pounds. Their unique feature is that they are the only bird with their nostrils located at the end of their long, slender beak. This

gives them the ability to smell earthworms, their primary food source, well in the forest litter. Kiwis are nocturnal and have poor eyesight, relying heavily on their sense of smell and tactile perceptions. The kiwi is also noted for producing the largest egg relative to its body size; the kiwi egg is equivalent to 10% of the hen's body size.

3,4

The tinamous are small, ground-dwelling birds from Central and South America that have recently been classified as ratites. They are found in the Order Tinamiformes and there are 9 genera and 46 species. Tinamous are believed related to the rheas through a common ancient ancestor, although their size and superficial appearance are more that of a quail or partridge. Their diets consist of fruits, seeds, insects and green plant shoots and buds. Their habitats vary from lowland forest and pampas to high-altitude forests and grasslands at 14,000 feet elevation.<sup>4</sup>

TABLE 1

Classification of Ratites

<b>Taxonomic Order/Family</b>	<b>Common Name</b>	<b>Native Location</b>
Order Struthioniformes	ostriches	Africa
Order Casuariiformes		
Family Casuariidae	cassowaries	Australia, New Guinea
Family Dromiceidae	emu	Australia
Order Rheiformes	rheas	South America
Order Apterygiformes	kiwis	New Zealand
Order Tinamiformes	tinamous	Central & So. America

Only a few ratites species are considered of economic importance. The ostrich is probably of the most economic significance, as its products are meat, leather and feathers. The emu would rank second in economic importance with its products of meat, fine oil and leather. The rhea ranks a distant third as a producer of exotic skins, and is primarily kept by hobbyists. The cassowaries, kiwis and tinamous are kept by zoos and other exhibition collections.

If there is to be a self-sustaining, profitable ratite industry, increased production is necessary. This will require research into the most appropriate nutrition, husbandry and medical management for the various species. At this time, juvenile morbidity and mortality are primary factors that need attention.

Toward this end, several diagnostic cases in ratites were investigated in an attempt to determine the cause of a degenerative myopathy affecting primarily juvenile ratites. History, gross necropsy and histopathologic examination of tissues were important factors in making the diagnosis of degenerative myopathy. History, examination of the diet, determination of mineral and vitamin levels in tissues, and response to therapy were also important factors in attempting to determine the cause of this myopathy.

This research project will describe the results of investigations into several cases of degenerative myopathy in ratite species, including one case where the response to treatment is presented. This is followed up by a survey of blood and dietary selenium and plasma and dietary vitamin E levels in clinically normal ratites, with comparisons being made between these normal birds and affected birds. The

practical goal is to arrive at some idea of what is a reasonable dietary level of selenium and vitamin E for ratites and to also provide some parameters for determining the cause of degenerative myopathy in ratites post-mortem.

## LITERATURE REVIEW

This research has three objectives. The first objective is to describe the gross and histopathologic lesions of degenerative myopathy in ratites via presentation of several clinical diagnostic cases. The second objective is to describe a case report of degenerative myopathy in a group of emus and their response to treatment. The third objective is to survey the blood selenium and plasma vitamin E levels of clinically normal ratites and compare these to the levels in clinically affected ratites. Where possible, the dietary levels of vitamin E and selenium are also examined and compared.

This review of the literature will focus on the possible causes of degenerative myopathy in ratites in particular, but also in other birds. There are a number of possible causes of degenerative myopathy in birds. Capture myopathy, deficiencies of selenium and/or vitamin E, furazolidone toxicity, ionophore toxicities, fusariomycotoxicosis and ingestion of the toxic plant, *Cassia spp.* are all possible causes of degenerative myopathy that need to be considered. In addition, other problems, such as genetic or inherited myopathies should at least be considered, although none have been documented in ratites as yet.

This thesis will also include a review of selenium and vitamin E deficiency in poultry, since historically these deficiencies have been a major cause of degenerative myopathy in poultry. Extensive research has been performed on the need for vitamin E and selenium in the diets of poultry, but little is known regarding these

nutrients in ratites and other species. It has been assumed by many of the feed companies that are formulating ratite diets that their dietary requirements for vitamin E and selenium are similar to those of domestic poultry.

### **History of Vitamin E and Selenium**

Tocopherols derived from plant lipids were found to support normal gestation in rats in the 1920's and later were also found to support normal vascular function in chicks. These tocopherols were recognized as natural antioxidants and accordingly were identified as "Vitamin E". A nutritional role for selenium was suggested in 1957, when it was found that selenium could replace vitamin E in the diets of rats to prevent hepatic necrosis and in the diets of chicks to prevent exudative diathesis. In the 1960's many investigators found that selenium could prevent or ameliorate the effects of vitamin E deficiency disorders, such as nutritional myopathies, reproductive failures, hepatic degeneration, vascular disorders and growth retardation, in multiple species. Since vitamin E was believed to act as a biologically specific lipid antioxidant, the nutritional interactions of selenium and vitamin E were felt to be evidence that selenium also had an antioxidant function. However, the mode action of selenium remained unclear and there was controversy regarding its true role in nutrition. Some investigators believed that selenium was a nutrient in its own right, while others believed that selenium was only a factor that produced a sparing effect on the function of Vitamin E.

In the 1970's, two findings helped to settle this controversy. First it was determined that a syndrome known as nutritional pancreatic atrophy of chicks could be produced as a result of selenium deficiency, even in chicks fed adequate amounts of vitamin E. Second, selenium was found to be an essential constituent of glutathione peroxidase, an enzyme with antioxidant function. These two findings and associated research established that selenium was an essential dietary nutrient and that its metabolic functions were intimately related to the functions of Vitamin E.

### **Modes of Action of Vitamin E and Selenium**

Vitamin E and selenium function as key elements of a system containing multiple components that provide antioxidant protection for cells.<sup>5</sup> Hydrophobic elements, such as vitamin E, carotenoids and ubiquinones, reside within cellular membranes. Hydrophilic elements, such as ascorbic acid, glutathione and glutathione peroxidase, reside in the cytoplasm and mitochondrial matrix space or the aqueous phase. The various system components function interactively to reduce free radicals that are generated by intracellular metabolic processes. They do this by preventing the initiation of oxidative processes, such as the oxidation of sulfhydryl groups on critical proteins and the peroxidation of polyunsaturated membrane phospholipids. These oxidative processes are known to lead to impairment of normal cellular function. Vitamin E, as the major membrane-bound lipid antioxidant, and selenium, as a necessary component of at least four forms of glutathione peroxidase (the cytosolic, phospholipid hydroperoxide, plasma and gastrointestinal forms), serve as

key elements in this cellular antioxidant system. Due to the phenolic character of its chromanol hydroxyl group, vitamin E serves as a reductant of free radicals. The antioxidant activity of vitamin E is determined by the positions and number of methyl groups on its chromanol ring. The configuration of the side chain determines the biologic specificity of the each particular vitamin E.<sup>5</sup>

Glutathione peroxidase is an essential enzyme that catalyzes the glutathione-dependent reduction of hydroperoxides, such as hydrogen peroxide and fatty acid hydroperoxides. Each mole of glutathione peroxidase contains four atoms of selenium; each selenium atom is present as a selenocysteine molecule at the active center of the enzyme.<sup>5</sup>

Selenium plays an important role in thyroid hormone metabolism. As part of the selenocysteine molecule in type 1 iodothyronine-5'-deiodinase, selenium is involved in the extrathyroidal conversion of thyroxine (T4) to metabolically active tri-iodothyronine (T3).<sup>6,7</sup> In humans and chickens, iodothyronine-5'-deiodinase in liver and muscle is responsible for approximately 80% of the T3 production. Evidence suggests that selenium deficiency is a factor in the etiology of iodine deficiency disease syndromes in human populations with sub-optimal iodine intakes.<sup>8,9</sup> Impaired endogenous heat production could be expected in selenium deficient animals, which then contributes to the reduced efficiency of growth and feed utilization commonly seen in these animals.

As other selenocysteine-containing proteins are described, more physiologic functions related to selenium may be discovered. Since the clinical signs of selenium

deficiency appear to be associated with the loss of iodothyronine-5'-deiodinase and/or glutathione peroxidases, the loss of other selenocysteine-containing proteins may also reveal themselves in changes in the animal's physiologic state.

### **General Signs of Selenium/Vitamin E Deficiencies**

Several organ systems in several species can be affected by deficiencies of vitamin E and/or selenium. The organ system affected can depend on a number of factors, including the presence or absence of other dietary factors, such as antioxidants, pro-antioxidants and sulfur-containing amino acids. Although clinical signs may vary among affected species, microvascular lesions causing local hypoxia appears to be a common pathogenesis. A great deal of basic physiologic and nutritional research involving vitamin E and selenium has been conducted with poultry as the experimental animal. Deficiency syndromes have been investigated in chickens, turkeys, ducks and Japanese quail.

### **Chickens**

Four distinct pathologic conditions have been described in domestic chickens related to selenium and/or vitamin E deficiency. These include encephalomalacia, exudative diathesis, nutritional muscular dystrophy and nutritional pancreatic atrophy. Impaired immunocompetence and reproductive failure (reduced egg production, decreased hatchability and reduced embryonic growth) have also been found related to deficiencies of selenium or vitamin E.

Encephalomalacia occurs in young chicks. Clinical signs include ataxia and depression; it is known as “crazy chick disease”. Grossly, pinpoint hemorrhages and edema may be seen on the surface of the cerebellum and in its parenchyma. The microscopic lesions are primarily found in the cerebellum and are characterized by hemorrhages and edema of the parenchyma related to microvascular injury. Severe ataxia can be produced by vitamin E deficiency in chick diets that contain no synthetic antioxidants. Concurrent selenium deficiency may cause exacerbation or acceleration of signs in chicks that are already vitamin E- and antioxidant-deficient.<sup>10</sup> Combs & Hady believe that this effect is due to the role of selenium in the utilization of vitamin E by neural tissue, since they have found normal brain concentrations of selenium and normal brain glutathione peroxidase activity in affected chicks.<sup>11</sup>

Exudative diathesis (ED) in chicks is characterized by severe subcutaneous edema, (especially of the abdomen, feet and ventral aspects of the neck and wings), anemia and hypoproteinemia. Increased capillary permeability is the cause of the edema. The edema then progresses to hemorrhage into the subcutaneous spaces. Affected chicks are depressed and show reduced activity and food intake. If untreated, affected chicks die within a few days. In vitamin E-deficient chicks, the condition is prevented by providing selenium in the diet. Combined deficiencies of both selenium and vitamin E produce exudative diathesis in young chicks within 2 to 4 weeks when they are raised on deficient diets from hatching. The selenium deficiency manifests itself by drops in glutathione peroxidase levels to less than 15% of controls within 6 days, but the initial signs of exudative diathesis are not yet

observable. By 2 to 4 weeks on the deficient diet, hepatic glutathione peroxidase has decreased to less than one-third that of controls, and erythrocyte glutathione peroxidase levels also show significant declines.<sup>12</sup>

Plasma glutathione peroxidase activity is inversely correlated to the risk of young chicks developing exudative diathesis. The direct involvement of glutathione peroxidase in exudative diathesis was proven by potentiating the disease through the use of glutathione peroxidase inhibitors, such as aurothioglucose and penicillamine, and by preventing the disease through the use of a synthetic selenium-containing compound, ebselen, which has glutathione peroxidase-like enzymatic activity.<sup>12</sup>

Both the rate and incidence of development of exudative diathesis is dependent on the vitamin E and selenium status of the flock. Selenium and vitamin E-depleted hens produce chicks that develop exudative diathesis within 6 to 12 days from hatch when raised on a low selenium, vitamin E-free diet. Hens that are adequately nourished produce chicks that develop exudative diathesis much later, when fed the same low selenium, vitamin E-free diet. Chicks from well-nourished hens, but fed marginal levels of vitamin E and/or selenium have a lower incidence of exudative diathesis than those from deficient hens or those fed a low selenium, vitamin E-free diet from hatch.<sup>12</sup>

Generalized muscular weakness and marked decreases in activity are the primary clinical signs of nutritional muscular dystrophy (NMD) in chicks. Degeneration of skeletal muscle is the primary lesion in NMD. Grossly, longitudinal white streaks may be found in various skeletal muscles, but are most prominent in the

pectoralis and gastrocnemius muscle groups. Histologically, there is Zenker's degeneration of myofibers with perivascular infiltration with macrophages; fibrosis is seen in sites attempting to repair damage. The dystrophic muscles show elevated activities of transaminases and lysosomal enzymes. When day-old chicks are raised on a dystrophigenic diet, myopathy is seen within 20 to 25 days. NMD in chicks is produced only when diets are moderately deficient in the sulfur-containing amino acid, cystine.<sup>13</sup> Experimentally, the synthetic antioxidants are not capable of preventing development of NMD in the chick, but diphenyl-p-phenylamine diamine (DPPD) was found effective when supplemented at high levels in low-fat diets. When 4% lard was added to the dystrophigenic diet, the protective effect of DPPD was lost.<sup>14</sup> For this reason, low levels of synthetic antioxidants are added in vitamin E-deficient diets to prevent other vitamin E- and antioxidant-related disorders, such as encephalomalacia, which would otherwise be produced in chicks.

Dietary supplementation with selenium is effective in reducing, but not fully preventing NMD. Although supplementation with selenium markedly reduced the amount of vitamin E necessary to prevent myopathy in chicks, it did not affect the level of cystine necessary for similar protection.<sup>15</sup> This finding suggested that the role of selenium in NMD was only to improve the tissue utilization of vitamin E and that NMD was primarily a disorder of vitamin E and cysteine metabolism. More experimental information indicated that the activity of selenium-glutathione peroxidase and the concentration of reduced glutathione were significantly greater in muscle from dystrophic chicks compared to non-dystrophic chick even when each

chick was fed dystrophigenic diets supplemented with selenium.<sup>16</sup> This suggests that oxidative stresses were associated with either increased needs for or decreased utilization of the glutathione peroxidase system. This hypothesis is further supported by the discovery of low molecular weight proteins, believed to be the products of proteolysis, and two- to three-fold increases in the ratio of protein-bound disulfide:sulfhydryl content in dystrophic chick muscle.<sup>17</sup> This oxidation of muscle proteins in NMD suggests that the need for sulfur-containing amino acids by chicks deficient in vitamin E is related to an increased requirement for essential amino acids during the rapid turnover of muscle proteins. Reductions in both the conversion of cysteine to taurine and the transsulfuration of methionine to cysteine in chicks deficient in vitamin E explains why cysteine is much more effective in the rapid turnover of muscle proteins.<sup>18</sup>

Nutritional pancreatic atrophy (NPA) was first described in an experiment in which chicks were fed a low selenium (0.010 ppm) purified diet that was also supplemented with excess vitamin E (100 IU/kg). Chicks on this purified diet showed decreased exocrine pancreatic function and impaired utilization of dietary fats. Supplemental selenium was required for growth and survival.<sup>19,20</sup>

The pathogenesis of NPA was described in the severely selenium-deficient chick. The first lesion is vacuolation of the pancreatic acinar cells and hyaline body formation. This is followed by cytoplasmic shrinkage, loss of zymogen granules and dilation of the acinar lumina.<sup>21</sup> In the final stage, macrophages and fibroblasts infiltrate the pancreatic parenchyma and is termed severe peri-acinar fibrosis. The

disease was initially named “pancreatic fibrosis”, but was renamed “nutritional pancreatic atrophy” as a result of recognizing that selenium deficiency produced atrophy of the acinar cells and that fibrosis was a consequence of that atrophy.<sup>5,21</sup>

The histologic lesions are associated with progressive decreases in the activities of proteases and lipase. The decrease in lipase activity results in impaired digestion of dietary triglycerides, reduced formation of intestinal mixed micelles, generalized impairment of enteric absorption of dietary lipids, and steatorrhea. A secondary deficiency of vitamin E developed due to the failure of absorption of fat-soluble vitamins. NPA did not affect the islets of Langerhans; selenium deficient chicks had normal plasma glucose concentrations and no signs of impaired pancreatic endocrine function.<sup>5,21</sup>

NPA was also described in second-generation selenium-deficient chicks.<sup>22</sup> The initial appearance of abnormal acinar cell morphology was usually observed within 4 to 6 days after hatching when chicks were raised on a selenium-deficient, vitamin E-supplemented diet. At this early stage, the chicks appeared clinically normal, however, feed intake began to decline and the rate of gain in body weight was slightly depressed. Feed intake became markedly depressed and weight loss and poor feathering were observed by 6 to 12 days, which corresponded with shrinkage of acinar cell cytoplasm. Some chicks died at 12 to 15 days with prominent pancreatic acinar atrophy and mild periacinar fibrosis. By 28 days on the selenium-deficient diet, chick mortality approached 95%.<sup>22</sup>

When compared to first-generation selenium-deficient chicks, the onset of NPA occurred 5 to 7 days later.<sup>22</sup> Pancreatic acinar degeneration was reversible by selenium supplementation and supplemented chicks had an immediate increase in appetite, which coincided with histologic evidence of acinar regeneration within 1 to 2 days of treatment; within 2 weeks, return to normal gross appearance and nearly normal histologic appearance was observed.<sup>22</sup>

The early phase of NPA, specifically in the chick, is associated with decreased rates of RNA and protein synthesis.<sup>23</sup> Pancreatic RNA and protein synthesis reverted to the normal rates of synthesis within 12 hours when deficient chicks were treated with selenium. It was hypothesized that this decreased synthesis was related to the altered function of acinar mitochondria, but the experimental results indicated that mitochondrial respiratory function in the chick pancreas was not affected by nutritional selenium deficiency.<sup>23,24</sup> Electron microscopy demonstrated disappearance of endoplasmic reticulum in degenerating pancreatic acinar cells, thereby providing a cause for decreased synthesis of RNA and protein.<sup>25</sup>

The depression of growth rate seen in severe, uncomplicated selenium deficiency in the chick results, at least in part, from depression of appetite. It was found that force-feeding chicks to levels of intake comparable to the *ad lib* intake levels of selenium-adequate chicks could overcome approximately two-thirds of the growth depression in chicks with NPA.<sup>26</sup> Growth was also promoted in selenium-deficient chicks by dietary supplements of cystine, but supplements of methionine were ineffective.<sup>27</sup> This finding was confirmed in certain strains of chickens in

subsequent studies. From these studies, selenium-deficient chicks with NPA appear to have either an increased metabolic need for cysteine which cannot be satisfied by transsulfuration from methionine, or an impairment in the transsulfuration pathway itself<sup>28</sup> Findings that selenium-deficient chicks had depressed plasma concentrations of homocysteine, cystathionine and cysteine<sup>23</sup> and increased rates of methionine-methyl group oxidation are evidence that metabolic conversion of methionine to cysteine is inadequate.<sup>29</sup>

NPA was produced on a corn-soy based practical diet containing less than 0.01 ppm selenium. This deficient diet produced only a very slight growth depression after 30 days, despite production of prominent pancreatic atrophy. This study suggested that there was some unidentified factor in the practical diet, other than cysteine, that prevented the severe growth depression seen with selenium deficiency studies using purified diets.<sup>30</sup>

Although NPA was considered to be the only clear-cut pathologic condition resulting from selenium deficiency, uncomplicated by deficiencies of vitamin E and cysteine, it was found that NPA could also be prevented by dietary supplementation with high levels of vitamin E, BHT, DPPD, ethoxyquin or ascorbic acid.<sup>31</sup> Chicks consuming diets containing at least 0.05 ppm selenium maintain normal pancreatic function, but NPA was also prevented by dietary supplementation with at least 300 IU vitamin E per kg of feed or by supplementation with 500 ppm of any of the synthetic antioxidants (BHT, DPPD, ethoxyquin) or ascorbic acid. Each of these treatments was capable of producing normal pancreatic histology and normal growth

in chicks on a diet deficient in selenium. The fact that supplementation with antioxidants did result in an increased utilization of trace amounts of dietary selenium was evidenced by the lack of increased glutathione peroxidase activity in plasma, pancreas or liver and by the lack of increased selenium content in the pancreas. This information led to the conclusion that, although NPA is exceedingly responsive to very low dietary concentrations of selenium, the disorder actually involves the total antioxidant status of the chick in a more general way.<sup>32</sup>

It has also been discovered that impaired development of immunocompetence occurs in selenium-deficient chicks. Chicks deficient in either selenium or vitamin E during the first 14 days after hatching show impaired humoral response to ovine erythrocytes. Fortunately, if selenium or vitamin E are replaced in the diet by 21 days of age, this immune response can be restored.<sup>32</sup> Studies have shown lesions of the bursal epithelium and depletion of lymphoid cells in chicks deficient in vitamin E and selenium, which may explain the reduced B-cell function observed in combined deficiency syndromes. These results suggest that vitamin E and/or selenium deficiency affect the resistance to disease in young, growing chicks.<sup>13</sup>

Reproductive failure in breeding chickens has also been described related to deficiencies of selenium or vitamin E.<sup>13</sup> Reduced rates of egg production and embryonic survival were produced in hens fed a corn-soy based diet containing less than 0.03 ppm of selenium and without supplementation of vitamin E. Following three weeks of feeding a supplemented diet, containing 0.10 ppm of selenium as  $\text{Na}_2\text{SeO}_3$  to the hens, the rates of egg production and embryonic survival returned to

normal; eggs contained an average of 0.121 ppm of selenium, and there was a protective effect against exudative diathesis in the progeny of these hens, even when the progeny were raised on a diet deficient in both selenium and vitamin E.<sup>33</sup> Additional studies concluded that a dietary requirement of approximately 0.05 ppm selenium was needed to maintain egg production in laying hens,<sup>34</sup> but that dietary levels of less than 0.10 ppm of selenium resulted in deficiencies of selenium-glutathione peroxidase in hens, impaired hatchability and impaired performance of progeny post-hatching.<sup>35</sup> A high incidence of late stage embryonic mortality with subcutaneous hemorrhages was seen in the progeny of hens deficient in vitamin E and selenium. Impaired testicular maturation in cockerels has been reported in uncomplicated selenium deficiency; testicular degeneration has been reported in roosters that are chronically vitamin E deficient.<sup>13</sup>

### **Turkeys**

Nutritional muscular dystrophy also occurs in selenium-deficient turkey poults and has been proven to be prevented by dietary supplementation with selenium.<sup>36</sup> This muscular dystrophy in poults is characterized by a pale gross appearance of the gizzard muscle and by hyaline degeneration of the myocardium, skeletal muscle and smooth muscle of the gizzard. Increases in serum transaminase activities and decreases in hematocrit, blood hemoglobin and albumin concentrations are associated with this myopathy, as well as decreased activities of selenium-glutathione peroxidase in multiple tissues.<sup>37</sup> An interesting contrast between the chick and the poult has been

observed. Although the skeletal muscle myopathy in the vitamin E-deficient chick can be prevented by dietary sulfur-containing amino acids, the gizzard myopathy of selenium- and vitamin E-deficient turkey poults is not. The gizzard myopathy of poults is completely prevented, however, by dietary supplementation with selenium, but the level of vitamin E in the diet influences the amount of selenium needed to prevent gizzard myopathy. One study indicated that a basal diet, low in methionine and vitamin E, as well as selenium, was necessary to experimentally produce gizzard myopathy.<sup>38</sup> Another study found that although a level of 0.18 ppm of selenium in the diet was necessary to prevent gizzard myopathy in poults fed adequate amounts of vitamin E, a higher level (0.28 ppm selenium) was necessary when the diet was not supplemented with vitamin E.<sup>39</sup> In studies where methionine and vitamin E were added to dystrophigenic diets, improved growth was seen but the incidence of gizzard myopathy was unaffected. In the hereditary skeletal myopathy of turkeys (deep pectoral myopathy), selenium, vitamin E nor methionine were capable of preventing the disease.<sup>40</sup>

A combined deficiency of vitamin E and selenium has been shown to produce exudative diathesis in turkey poults, characterized by hemorrhages in the breast and thigh musculature. This is in contrast to only a mild edema in these locations in chicks with exudative diathesis. A macrocytic anemia and hydropericardium were also seen in poults affected by exudative diathesis.<sup>13</sup>

## Ducks

Combined deficiencies of selenium and vitamin E produce nutritional muscular dystrophy in ducks. Degeneration of the mitochondria and sarcoplasmic reticulum of the smooth muscle of the gizzard and duodenum were the first observed ultrastructural lesions.<sup>41,42</sup> The dystrophic gizzard smooth muscle exhibits pale areas of necrosis upon gross examination, which is accompanied by mineralization of sarcoplasmic debris in necrotic cells and infiltration by macrophages and fibroblasts. Lesions of unmyelinated nerve fibers and capillaries were not seen subsequent to extensive myonecrosis. Lesions were also identified in the skeletal muscles and myocardium. At the light microscopy level, skeletal muscles have a hyalinized appearance with extensive lysis of myofibrils; mitochondrial swelling is observed ultrastructurally.<sup>41,42</sup>

NMD in the duckling can be prevented by supplementation of a corn-soy based practical diet containing 10 IU of vitamin E per kilogram of diet and 0.04 ppm of inherent selenium, by supplementation with 0.10 ppm selenium as  $\text{Na}_2\text{SeO}_3$ . However, it was also found that even higher levels of selenium were necessary to evoke maximal selenium glutathione peroxidase activity in both plasma and liver tissue.<sup>43</sup>

It was proposed that connective tissue metabolism was impaired in ducklings deficient in selenium and vitamin E.<sup>44</sup> Both soluble and total collagen were decreased in tendons from ducklings fed a practical diet low in vitamin E and selenium, when compared to tendons from ducklings fed a diet supplemented with 0.5

ppm selenium as  $\text{Na}_2\text{SeO}_3$ . The tendons from the selenium-deficient ducks contained degenerating fibroblasts. The findings from this study were interpreted as a failure of collagen maturation and it was hypothesized that this functional failure in tendons could lead to myofibrillar degeneration of muscle as seen with NMD.<sup>44</sup> This hypothesis was supported by the work of other investigators who also described structural alterations in the collagen of tendons from selenium-deficient ducks<sup>45</sup> and discovered the protective effects of supplementation with ascorbic acid (a factor involved in collagen synthesis and metabolism) in selenium deficiency.<sup>44,46,47</sup> However, this response to ascorbic acid supplementation was more likely due to its antioxidant effects than due to correction of impaired ascorbic acid biosynthesis.<sup>43,48</sup> Indeed, it was found that supplementation of poultry diets with high levels of ascorbic acid enhanced the utilization of dietary selenium.<sup>48</sup>

Exudative diathesis has also been reported in conjunction with NMD in ducklings deficient in selenium and vitamin E. The condition in ducklings is similar to that of the chick: greenish edema of subcutaneous tissues and hemorrhage of musculature, primarily of the thigh. The appearance of exudative diathesis is infrequent, however, and seems to occur only in the most severe cases of NMD.<sup>49</sup>

### **Japanese Quail**

Young Japanese quail raised on a diet deficient only in selenium exhibited reduced feed consumption, severely depressed growth, poor feathering and poor survival.<sup>50</sup> A combined deficiency of selenium and vitamin E produced exudative

diathesis in some animals. Although egg production and fertility were not affected by a combined deficiency of selenium and vitamin E, hatchability and embryonic survival were markedly depressed in eggs from quail hens that were raised to maturity on deficient diets.<sup>51</sup> In addition, most of the surviving progeny from these selenium and vitamin E-deficient quail hens exhibited severe generalized muscular weakness post-hatching. Embryonic survival was returned to normal following supplementation of the quail hens with either selenium or vitamin E; quail hen mortality was also reduced by this supplementation.

A summary of the disease syndromes in poultry is provided in Table 2.

TABLE 2

Selenium/Vitamin E Deficiency in Poultry<sup>13</sup>

Species/Syndrome	System affected	Prevention by	
		Selenium	Vitamin E
<b>Chicken</b>			
Exudative diathesis	capillaries	+	+
Muscular dystrophy	skeletal muscle	+ <sup>a</sup>	+ <sup>b</sup>
Pancreatic atrophy	acinar pancreas	+ <sup>c</sup>	+ <sup>d</sup>
Encephalomalacia	cerebellum	?	+ <sup>c</sup>
Impaired immunity	bursa	+	+
Reduced egg production	ovary	+	+
Decreased hatchability	embryo	+	+
Reduced growth	chick	+	+
<b>Turkey</b>			
Muscular dystrophy	skeletal/smooth muscle	+	+ <sup>a</sup>
Exudative diathesis	capillaries	+	+
Reduced growth	poult	+	+ <sup>b</sup>
<b>Duck</b>			
Muscular dystrophy	skeletal/smooth muscle	+	+
Connective tissue	tendon fibroblasts	+	+
Exudative diathesis	capillaries	+	+
<b>Japanese quail</b>			
Exudative diathesis	capillaries	+	+
Reduced hatchability	embryo	+	+
Reduced growth, survival	offspring	+	+

<sup>a</sup> Provides only partial protection

<sup>b</sup> Partial protection is provided by cyst(e)ine

<sup>c</sup> Disorder exacerbated by high polyunsaturated fatty acid (PUFA) levels

<sup>d</sup> Protects at only very high levels

## **Selenium Bioavailability**

Not all dietary selenium is absorbed, and not all absorbed selenium is retained in metabolically active forms. Experimental animal studies have demonstrated several factors that can affect the net physiological utilization of selenium. These factors may include the chemical form of selenium, the bioavailability of that form, the presence or absence of other feed components, the presence or absence of antioxidants, and the total diet composition. Generally, the inorganic salts of selenium, sodium selenate ( $\text{Na}_2\text{SeO}_4$ ) and sodium selenite ( $\text{Na}_2\text{SeO}_3$ ) tend to be highly available forms of selenium. These same forms are also susceptible to reduction to very poorly available or insoluble forms, such as selenides and elemental selenium. This susceptibility to reducing conditions probably accounts for apparently increased needs for total dietary selenium.

Factors that are known to antagonize selenium bioavailability include heavy metals, sulfates, mercaptans, chlorinated hydrocarbons and deficiencies of vitamin E, riboflavin, vitamin B6 and methionine. Factors that tend to spare or enhance selenium bioavailability include vitamin E, restricted food intake, and high levels of vitamin A, vitamin C and synthetic antioxidants.<sup>13</sup>

Food sources of selenium vary in their bioavailability. Meat sources tend to vary, but generally have a fair to good utilization in experimental situations. Meat sources exhibit a 60 to 90% bioavailability compared to sodium selenite. Plant sources of selenium vary widely in their bioavailability. Most plant sources exhibit a moderate value of 40 to 80% when compared to sodium selenite, while other plant

sources exhibit bioavailability superior to inorganic selenium salts. Selenium bioavailability is not simply a property of feedstuffs themselves, but rather it is markedly affected by the total diet composition. In most situations, the bioavailability of dietary selenium from any source can be enhanced through appropriate utilization of antioxidants.<sup>13</sup>

### **Recommended Dietary Levels of Selenium and Vitamin E for Poultry**

Levels of vitamin E and selenium in practical poultry diets are presented in Table 3.

TABLE 3

Recommended Dietary Levels of Vitamin E and Selenium  
for Practical Poultry Diets<sup>13</sup>  
(for total contents of each nutrient in finished feeds)

Species	Vitamin E IU/kg	Selenium mg/kg
Chicken		
Starting & growing chicks	7.5	0.15
Laying hens	10	0.10
Breeding hens	20	0.15
Turkey		
Starting & Growing poults	5	0.20
Laying hens	5	0.15
Breeding hens	10	0.20
Geese		
Starting & Growing	5	0.20
Laying & Breeding	10	0.20
Ducks		
Starting & Growing	5	0.15
Laying & Breeding	10	0.15
Pheasants, bobwhite quail and Japanese quail		
Starting & Growing	7.5	0.15
Laying & Breeding	20	0.15

In the case of naturally occurring tocopherols, it is difficult to predict with any certainty the actual vitamin E content of practical feeds because of the wide variation in the amount of natural tocopherols and their intrinsic instability. For this reason, supplementation of practical diets with stable, highly bioavailable forms of vitamin E are required to ensure adequate dietary levels.<sup>13</sup>

Most practical feeds, especially high protein content feeds, can be predicted to contain substantial quantities of selenium, except in areas of endemic selenium deficiency. Most corn-soy based diets contain at least 0.05 mg/kg, which is 25 to 33% of the desired amount. Supplementation with selenium at the rate of 0.1 to 0.15 mg of **added** selenium per kilogram of finished feed is usually necessary to ensure optimal poultry health and productivity.<sup>13</sup>

## **Myopathies in Ratite Species**

### **Myopathies Attributed to Deficiency of Vitamin E or Selenium**

A review of the literature reveals few references to myopathies of any kind in ratite species. In a case report from South Africa, vitamin E/selenium deficiency was suspected in two 4-month-old ostriches with paresis of the hindlimbs and elevated serum levels of aspartate transaminase (AST) and creatine kinase (CK). The diet consisted primarily of crushed maize. A single injection of a vitamin E/selenium preparation (Bo-Se; Cyanamid) resulted in the recovery of one of these ostriches, but the other died despite therapy. At necropsy, focal pale regions of the thigh

musculature were observed. Microscopic examination revealed degeneration, necrosis and regeneration of skeletal muscle fibers, as well as fibrinoid degeneration and necrosis of some arterioles and varying degrees of interstitial fibrosis.<sup>52</sup>

In another case report from South Africa, six 6-week-old ostrich chicks on a diet of alfalfa hay developed lameness. Two of the chicks died. Pathological examination of one of the dead chicks revealed lesions consistent with nutritional myopathy in the larger muscle groups. Therapy with an injectable vitamin E/selenium preparation (Injacom E-Selenium, Roche) cured the other four chicks.<sup>53</sup>

Not all instances of degenerative myopathy can be attributed to deficiencies of vitamin E or selenium. Degenerative changes in the musculus complexus (hatching muscle) and the muscles of the pelvic limbs were accompanied by anasarca in 20 ostrich chicks, dying 1 week post-hatching in Australia. Biochemical examination of the tissues did not show evidence of deficiencies in selenium, vitamin E or vitamin A. This syndrome in very young ostrich chicks was attributed to high relative humidity during incubation and malpositioning, which resulted in anasarca and an exertional myopathy related to difficulty during the hatching process.<sup>54</sup>

### **Selenium Toxicity**

Although there is concern in the ratite industry regarding selenium/vitamin E deficiency as the cause of degenerative myopathy, selenium oversupplementation should be an equal concern because of the narrow therapeutic range of selenium. There is a solitary report of apparent selenium toxicity in a flock of emus (*Dromaius*

*novaehollandiae*).<sup>55</sup> Markedly decreased production was experienced in this commercial emu breeder flock during the breeding season. The decreased production was characterized by a decrease in the number of eggs laid by hens, decreased fertility and increased embryonic death resulting in an overall decrease in hatchability. The diet was a mixture of catfish food supplemented with a vitamin E and selenium premix. When the diet was analyzed, it was found to contain 1.55 ppm selenium. The National Research Council recommends a level of 0.15 ppm selenium for chicken broilers.<sup>56</sup> Levels of 2 ppm in feed have been found to be toxic to chickens.<sup>57</sup> Eggs that failed to hatch were analyzed for a variety of constituents, including selenium. Egg selenium levels while the hens were on the supplemented feed ranged from 1.2 to 7.1 ppm (mean  $4.2 \pm 0.7$  ppm).<sup>55</sup>

Bird embryos are extremely sensitive to the toxic effects of selenium. Field studies in wild waterfowl have shown that sampling of eggs is a good measure of selenium bioavailability and the potential for adverse effects on reproductive performance. Hatchability of fertile eggs is considered the most sensitive measure of selenium toxicity, primarily because selenium seems to preferentially accumulate in eggs and liver. Fertility may not be affected, but high selenium results in high rates of embryonic mortality and teratogenic effects, such as deformities of the eye, limbs, beak, brain and abdomen. Among stilt (*Himantopus mexicanus*) eggs monitored for reproductive success or failure, an egg containing greater than 4.2 ppm selenium was four times more likely to fail to hatch than an egg containing less than that amount.<sup>58</sup> Selenium levels greater than 2.5 ppm in whole eggs are considered toxic in poultry.<sup>59</sup>

### Vitamin E Toxicity

Vitamin E is not generally considered toxic even when consumed in large amounts. The presumed maximum safe dietary vitamin E level for poultry chicks is 1000 IU/kg of diet on a dry matter basis.<sup>60</sup> Cases of apparent toxicity attributable to oversupplementation with vitamin E are rare in the literature. These invariably occurred in captive situation in species where vitamin E supplementation is considered not only appropriate, but necessary. In a group of captive white-tailed sea eagles (*Haliaeetus albicilla*), early embryonic death, poor egg hatchability, high chick mortality and histologic lesions consistent with vitamin E deficiency (pipping muscle edema, myodegeneration and mineralization) were reported in spite of markedly elevated serum  $\alpha$ -tocopherol levels ( $670.8 \pm 94.6 \mu\text{g/ml}$ ;  $n=4$ ) in the adult breeding birds. The diet of these piscivorous birds was heavily supplemented with vitamin E and when analyzed was found to contain 925-1364 IU/kg of diet, dry matter basis. None of the other raptors (non-piscivorous) at the same facility were affected; their diet contained vitamin E at a level of 48 IU/kg and no mean serum  $\alpha$ -tocopherol levels were above  $127 \mu\text{g/ml}$  ( $n=30$ )<sup>61</sup>

A coagulopathy in pink-backed pelicans (*Pelecanus rufescens*) has also been associated with hypervitaminosis E. Coagulopathies in the living birds were corrected by parenteral vitamin K therapy. Analysis of the diet indicated that these piscivorous birds were receiving an average vitamin E supplement of 9600 IU/kg of fish (dry matter basis) every other day. Liver  $\alpha$ -tocopherol levels ranged between 244 and 500  $\mu\text{g/g}$  of liver (dry weight); this was considered to be 5-25 times the

normal range of avian hepatic  $\alpha$ -tocopherol concentrations. High levels of vitamin A and D were also found in the supplemented diet. It was speculated that excessive amounts of fat-soluble vitamins A, D and E may interfere with the intestinal absorption of vitamin K by occupation of all the carrier proteins for fat soluble vitamins.<sup>62</sup>

Vitamin K-responsive coagulopathies due to chronic ingestion of large amounts of vitamin E have been reported in man and birds<sup>63,64</sup>. The exact mechanism of the how vitamin E interferes with coagulation is not known. In vitro studies suggest that quinone metabolites of vitamin E may competitively inhibit the function of vitamin K.<sup>65,66,67</sup> Excessive vitamin E supplementation in young, growing chickens is known to depress growth and pigmentation, and increase requirements of vitamin A, D and K.<sup>56</sup>

### **Exertional or Capture Myopathy in Ratites and Other Avian Species**

Capture myopathy has been reported in numerous wild and domestic mammalian species. The reports in wild or domestic birds are few. Although the incidence of capture myopathy is suspected to be high in ratites, there is only a solitary case report of the condition in the literature. This case report involved a 20-month-old emu (*Dromaius novaehollandiae*) that was found hanging by its right toe from the top of a 6 foot fence after being observed fighting with other bird. Serum aspartate transaminase (AST) and lactate dehydrogenase (LDH) levels were markedly elevated. Despite aggressive treatment for suspected capture myopathy, the bird

failed to respond and was euthanized. At necropsy, rupture and hemorrhage of the adductor muscles of the hip and extensive hemorrhage in the sublumbar musculature were found. The swollen lateral thigh muscles exhibited areas of paleness and emphysema, as well as areas of greenish bruising. The histopathologic diagnosis was capture (exertional) myopathy characterized by widespread myofiber hyalinization, coagulation, fragmentation, granular degeneration, mild mineralization, infiltration by macrophages, sarcolemmal and satellite cell proliferation, perifibrillary cellularity and capillary prominence; severe hemorrhage was present in the muscles of the hip and the sublumbar muscles.<sup>68</sup>

Exertional or capture myopathy has also been reported in the cranes,<sup>69,70,71</sup> storks,<sup>72</sup> flamingos,<sup>73</sup> wild waterfowl,<sup>74,75,76,77</sup> wild turkeys,<sup>78</sup> gulls and eagles.<sup>79</sup> An excellent review of the affected species, clinical signs, clinical pathology, microscopic pathology, etiology and pathophysiology of capture myopathy is presented by Williams and Thorne.<sup>79</sup> Considerable confusion in the literature exists concerning nutritional myopathies and exertional myopathy. The gross and histologic lesions are very similar and may require specialized techniques for differentiation between the two conditions. The clinical history is critical to the diagnosis of exertional myopathy.<sup>79</sup> Confusion continues as some cases of exertional myopathy are believed to be exercise-induced manifestations of nutritional myopathies.<sup>80</sup>

There is still some discussion regarding the pathophysiology of exertional myopathy. The underlying pathogenesis appears similar to that of shock and primarily mediated through the sympathetic nervous system.<sup>81</sup> Hyperthermia and

metabolic acidosis are also believed to be centrally important to the pathogenesis.<sup>82</sup> The metabolic acidosis is found to be more severe in animals chased rapidly over a short distance and less severe in more prolonged, but less intense, chases. It is postulated that the metabolic acidosis at the tissue level results in increased cell membrane permeability and lysis and release of intracellular electrolytes and enzymes, such as aspartate transaminase (AST) and creatine kinase (CK) into the blood stream. This increased vascular permeability and shift in electrolytes can then produce depressed cardiac output, falling blood pressure, cardiac arrhythmias and death. It is also postulated that heat and lactate generated locally in over-exerted muscles combine to produce the degenerative changes and necrosis of myofibers. Localized swelling and edema following this damage to the myocytes increases the local pressure on the muscles from the surrounding fascia, predisposing the muscles to ischemia, similar to that seen with “compartment syndrome”.<sup>79,80</sup> It has been suggested that lactic acid produced in muscles can cause both local and metabolic acidosis, resulting in the clinical signs and lesions of exertional myopathy in ducks.<sup>76</sup>

### **Myopathies in Other Species**

There are scattered case reports in the literature concerning degenerative myopathies in various non-domestic avian species. Degenerative myopathy has reportedly been a problem in captive piscivorous species of birds, such as pelicans, herons, penguins and other seabirds, where a pansteatitis may also be a manifestation of vitamin E deficiency.<sup>83,84,85,86,87,88,89</sup> This has prompted supplementation of the diets

of piscivorous birds with antioxidants and vitamin E. It is believed that storage of food fish results in fat rancidity problems, thereby increasing the need for antioxidants, such as vitamin E. Degenerative myopathy or steatitis may develop in piscivorous birds on diets low in vitamin E and high in long-chain polyunsaturated fatty acids (PUFAs). These unsaturated lipids are oxidized to peroxides by molecular oxygen, which is catalyzed by free radicals. Vitamin E is capable of breaking this chain of oxidation reactions by combining with the free radicals. However, vitamin E stores in the body can become depleted if the level of PUFAs is high in the diet.<sup>90</sup>

Degenerative myopathy believed related to vitamin E/selenium deficiency has been reported in raptors, sometimes also associated with poor reproductive capabilities.<sup>91,92,93</sup> Pink pigeons (*Nesoenas mayeri*) with cardiomyopathy diagnosed at necropsy were found to have undetectable plasma  $\alpha$ -tocopherol levels,<sup>94</sup> compared to nine normal pink pigeons that had mean plasma  $\alpha$ -tocopherol levels of  $12.39 \pm 1.73 \mu\text{g/ml}$ .<sup>83</sup>

Lesions of degenerative myopathy have been demonstrated in psittacines and wild anseriformes.<sup>95,96</sup> Myonecrosis of the smooth muscle of the gizzard was a common finding in affected ducks.<sup>96</sup> All-seed diets high in PUFAs, oversupplementation with cod-liver oil and diets low in vitamin E and/or selenium have been implicated in the lesion in these species.

Skeletal muscle damage was the most common histologic finding in wild sandhill cranes during a large-scale natural die-off. Investigators suspected

fusariomycotoxicosis as the cause of the die-off because trichothecene mycotoxins were recovered from *Fusarium*-infested peanuts that the cranes were feeding on.<sup>97,98</sup>

### **Toxic Myopathies**

Toxic myopathies are well documented in poultry, but no cases have been documented in ratites, as yet. The toxic myopathies have been associated with furazolidone (NF-180), ionophore coccidiostats and toxic plants of the *Cassia* genus.

Furazolidone (NF-180) is a nitrofurantoin antibiotic. Furazolidone toxicity affects primarily the heart and it has been reported in turkeys, ducks and chickens. There is a marked age susceptibility with juvenile birds being primarily affected. Furazolidone causes a dose-related dilatative cardiomyopathy resulting in congestive heart failure and death. Turkeys are particularly prone to furazolidone toxicosis, but the cardiomyopathy cannot be distinguished from spontaneous turkey cardiomyopathy, the cause of which is unknown. The microscopic lesions include edema, thinning of the cardiac myofibers, multifocal myocytolysis, and increased interstitial and endocardial fibrosis in subacute or chronic cases.<sup>99</sup>

Ionophores are ion carriers that facilitate the movement of cations, such as potassium, sodium, calcium and magnesium, across cell membranes. They also have anticoccidial activity because of their ability to preferentially move ions into the various stages of the coccidian parasites. The coccidiostats monensin, salinomycin, lasalocid and narasin are ionophores. Ingestion of toxic levels of ionophores cause potassium to leave the cells and calcium to enter the cells, especially myocytes,

thereby resulting in cell death. The microscopic lesions are primarily degeneration and necrosis of myofibers in the heart and skeletal muscle, indistinguishable from nutritional myopathy. Toxicity has been reported with ingestion of monensin, lasalocid, salinomycin and narasin in chickens, turkeys, guineafowl and quail.<sup>99</sup>

In the southern United States, pastures may contain the shrubs and trees of coffee senna (*Cassia occidentalis* or *Cassia obtusifolia*). The beans may be ingested, especially after a killing frost, which makes them more palatable. Ingestion of beans for a few days results in diarrhea, weakness, gait abnormalities and recumbency. The muscular lesions it produces cannot be distinguished from those of nutritional myopathy. The specific toxin has not been characterized. This intoxication has been documented in cattle and other ruminants.<sup>80</sup> Experimental intoxication has been reported in domestic chickens.<sup>100</sup> Although intoxication of ratites by this plant has not been documented in the literature, many ruminant pastures are being used for ratite production and this toxic plant ingestion should be considered in the differential diagnosis of acute degenerative myopathy.

### **Inherited, Spontaneous and Idiopathic Myopathies**

Inherited muscular disorders or myopathies have been described in several avian species, but not in ratites. These disorders include inherited muscular dystrophy of chickens, deep pectoral myopathy, focal myopathy of turkeys, acid maltase deficiency of Japanese quail and progressive myotonic dystrophy of “locked wing cross” Japanese quail (*Coturnix coturnix japonica*).

Inherited muscular dystrophy of chickens is characterized by atrophy of muscle fibers and progressive replacement by fat and connective tissue; the histologic lesions are usually distinct from nutritional myopathy. The condition is seen in inbred New Hampshire and Cornish strains of chickens and appears to be a single gene defect, but it is not known whether it is a point mutation or a deletion. The condition can be kept out of commercial flocks by genetic selection.<sup>101</sup>

Deep pectoral myopathy, also known as “green muscle disease” is primarily a problem in strains of adult meat-type turkeys and chickens that have been selected for heavy pectoral musculing. This condition was first described in Oregon in breeder turkey hens. It is a polygenic abnormality of the supracoracoideus muscle which predisposes it to ischemia and necrosis following exercise.<sup>101,102</sup>

Focal myopathy of turkeys is distinct from deep pectoral myopathy. It is characterized by finding degenerating myofibers in several muscles of the leg and breast and is often associated with leg weakness in strains of turkeys selected for rapid growth. It has been suggested that rapidly growing turkey muscle fibers outpace the growth of the blood supply and connective tissue support system. When the turkeys are stressed by capture to send them to market, localized regions of ischemia and loss of muscle glycogen occurs.<sup>101</sup>

Acid maltase deficiency in a strain of Japanese quail is a type II glycogenosis or glycogen storage disease.<sup>103</sup> Another mutant strain of Japanese quail, the “locked wing cross”, has recently been described. These birds have difficulty lifting their wings, therefore the name “locked wing cross”. The condition resembles human

myotonic dystrophy and is characterized by myofiber degeneration, testicular degeneration and lenticular cataracts.<sup>104</sup>

Ascites and right ventricular failure in broiler chickens (ARVF) is commonly caused by pulmonary hypertension in chickens raised at high altitudes.<sup>102</sup>

Spontaneous cardiomyopathy of turkeys is an idiopathic condition, previously known as round heart disease of turkeys and cardiohepatic syndrome. Some studies have shown a genetic predisposition in certain strains of turkeys. Dilatation of the ventricles and heart failure are the primary lesions.<sup>102</sup> Round heart disease of chickens is another idiopathic disease of the myocardium. The histologic lesions are characterized by cardiac myofibers that are dilated and vacuolated with prominent cell membranes; the lesions of round heart disease differ from those of nutritional myopathy.<sup>102</sup>

Hydropericardium-hepatitis syndrome (HHS) of chickens, also known as Angara disease, was originally thought to be caused by a toxicity or nutritional deficiency. Recently, however, the condition was experimentally reproduced by infection with a group 1 adenovirus. Hydropericardium, myocardial necrosis and hepatic necrosis with basophilic intranuclear inclusions in hepatocytes are presumptive evidence of HHS; confirmation of infection can be accomplished via virus isolation, electron microscopy and serum neutralization identification of the adenoviral agent.<sup>105</sup>

Recently, 17 cases of idiopathic rear-limb necrotizing skeletal myopathy were described in 4- to 10-week-old turkeys from California and Oregon. Muscles of the

legs and abdomen were affected; feed levels of selenium were adequate. Although the histologic lesions closely resembled those produced by ionophore toxicosis, the levels of monensin in the feed were within approved limits. These cases sparked concern about possible compounding or potentiating factors that may cause turkeys on approved levels of monensin to develop ionophore-related myopathy.<sup>106</sup>

## MATERIALS AND METHODS

### **Case Descriptions**

Nine cases of degenerative myopathy in ratites species were selected from diagnostic cases submitted to the Veterinary Diagnostic Laboratory at Oregon State University.

### **Survey of Clinically Normal Ratites**

Blood samples were solicited from practicing veterinarians or collected by the investigator. On farms where multiple birds were sampled, a feed sample that was currently being fed to those birds was also collected.

The blood samples were collected from clinically normal ostriches and emus with no clinical evidence of degenerative myopathy. The blood samples were collected either from the jugular vein or the ulnar vein, depending on the location most easily accessible to the venipuncturist. The blood sample from each bird was placed in two separate heparinized tubes: one for whole blood selenium determination and the other was centrifuged and the plasma collected for plasma vitamin E determination. Whole blood and plasma samples were frozen until shipped. The samples were either transported the same day to the laboratory or shipped overnight on ice in styrofoam containers. Samples were frozen until analysis.

Blood and feed samples were collected from a total of 75 ostriches from 9 different farms. Blood and feed samples were collected from a total of 62 emus from 6 different farms.

Whole blood was analyzed for selenium using an automated fluorometric method.<sup>107</sup> After thorough mixing on a rotator, 0.5 ml of whole blood was mixed with 7 milliliters of acid digestion mixture (concentrated nitric acid and concentrated phosphoric acid 4:3 v:v) in a Teflon microwave vessel. Vessels were capped and heated in the microwave oven for 8 minutes at 100% power. Vessels were cooled and caps removed. The vessel contents were emptied into weighed and labeled Erlenmeyer flasks using a 2 milliliter hydrogen peroxide rinse for each vessel. Flasks were placed on a hot plate (160 C°) and boiled until 3 to 4 milliliters remained. Flasks were cooled to room temperature and a second addition of 2 milliliters of hydrogen peroxide was placed in the flasks. Flasks were again heated on the hot plate and 6 milliliters of 50% concentrated hydrochloric acid and 2 milliliters of formic acid were added. Flasks were placed on the 130 C° hot plate for 10 minutes. After removal and cooling, 2.5 milliliters of hydroxylamine-EDTA solution was added and mixed. The pH was adjusted to 2.2 to 2.7 with 10N sodium hydroxide. Flasks were weighed. Samples were analyzed within 5 hours of pH determination.

The Technicon Autoanalyzer II system was warmed up and calibrated. The run consisted of the blank, standards and samples, followed by the standards and the blank again through the autoanalyzer. Fluorometric determinations were printed out on the recorder. The peak heights were measured. Final concentrations of the

standards were plotted against the peak heights to obtain the slope and intercept. The values were entered into the computer and using SuperCalc 4, and sample determinations are produced.

Plasma was analyzed for vitamin E using a modified high pressure liquid chromatography (HPLC) assay.<sup>108</sup> An extraction solution of acetonitrile, methanol, and chloroform (60:25:15, v:v:v) and 0.01% ascorbic acid, with 2.5 µg/ml of tocol as internal standard was used. Standards and samples were allowed to warm to ambient room temperature (20C°) while mixing on a rocker. A 200 microliter aliquot of sample or standard was added to 1.8 milliliters of extraction solvent in a 12 x 75-mm glass test tube. Each sample was mixed on a vortex mixer for 30 seconds, then centrifuged for 5 minutes at 2000G. The supernatant was pipetted into a 1 milliliter amber autosampler vial, and a blank cap with a Teflon insert was crimped on the vial. The standards were mixed on a vortex mixer for 15 seconds and directly added to the autosampler vials. Each sample was prepared and determined in duplicate, with all the samples from one bird determined together in a set. The standards at 20, 10, 5, 2.0, 1.0 and 0.5 µg/ml were analyzed at the beginning and end of the sample analysis, and a 2.5 µg/ml standard was determined after every tenth sample. An autosampler was used to inject the sample into the high pressure liquid chromatograph. The mobile phase was 100% methanol at a flow rate of 0.5 ml/min. A C-18 column (25 cm x 4.6 mm) was used with a C-18 (2 cm x 4.6 mm) precolumn. Fluorescence was quantitated, using an excitation wavelength of 295 nm with slit width of 15 nm, and an emission wavelength of 330 nm with slit width of 10 nm. The analysis time was

approximately 12 min/sample and the mean retention times for tocol and alpha tocopherol were 5.4 and 8.2 minutes, respectively. Quantitation was based on areas under the curves.

Feed samples were analyzed for vitamin E and selenium in a similar method as described for plasma and blood, following an extraction technique.

## RESULTS

### Case Descriptions

A significant number of young ratites (ostrich, emu and rhea) that were presented to the Oregon State University Veterinary Diagnostic Laboratory (OSU-VDL) exhibited histologic lesions of degenerative myopathy. Nine cases are summarized in Table 4: four in ostriches, three in emus, and two in rheas. The vast majority of these cases of degenerative myopathy occurred in ratite chicks six months of age or younger. Clinical history of illness in these birds was often scanty and clinical signs commonly consisted of simply acute death. A few chicks exhibited depression, weakness and inability to stand anywhere from a few hours to a couple of days prior to death. In most of these cases, ante mortem blood samples were not available. At necropsy, fresh liver was available in only a few cases and was submitted for selenium and vitamin E determination; this data is presented in Table 5.

In two cases, capture and/or transport occurred anywhere from five hours to two days prior to death. In the case involving the six-month-old rhea (Case 6), the bird was sold and transported a considerable distance. Following transport, it became progressively depressed, was eventually unable to rise or walk and died approximately two days after transport. White foci and streaks in the myocardium and muscles of the hindlimbs were described at necropsy. The histologic lesions in this rhea consisted of multiple regions of myocyte degeneration with infiltration of macrophages and early calcification. The gross and histologic lesions of severe

myocardial and skeletal muscle degeneration with calcification were consistent with those seen with nutritional myopathy or capture myopathy. This rhea's (Case 6) liver selenium levels was 0.175 ppm wet weight. Normal liver selenium levels for this species could not be located in the literature. Liver selenium levels below 0.25 ppm are considered deficient in poultry and levels below 0.35 ppm are considered marginally deficient.<sup>59</sup> The liver vitamin E level in this rhea was 0.69 µg/g of liver. Again, the meaning of this value is not clear, as normal liver vitamin E levels could not be located for this species or any other ratite, but was suspected to be below normal.

Case 4 involved a 10-month-old ostrich which had been sedated with xylazine prior to examination of the foot. The bird died very acutely five hours after xylazine administration, but had appeared perfectly normal 30 minutes prior to death. The submitted tissues showed acute severe pulmonary edema and acute myocardial degeneration.

The one-week-old ostrich chick (Case 2) had a liver selenium level of 0.495 ppm wet weight. The three-week-old emu in Case 7 had a liver selenium level of 0.822 ppm and a liver vitamin E level of 9.10 µg/g of tissue.

The emus from Cases 8 and 9 came from the same flock. The emu chick in Case 8 succumbed first, being found acutely dead. The owner had experienced mortality in several previous chicks of this age. The chicks became depressed, stopped eating and refused to stand or walk. Death ensued within four to five days after initial clinical signs. Lesions characteristic of degenerative myopathy were

TABLE 4  
Myopathy Lesions

Case	Species	Age	Sex	Lesion Location
1	ostrich	6 mo	F	heart muscle
2	ostrich	1 week	F	skeletal muscle
3	ostrich	1 week	?	skeletal muscle
4	ostrich	10 mo	M	heart; pulmonary edema
5	rhea	4 mo	M	skeletal muscle, gizzard muscle, fractured femur
6	rhea	6 mo	M	heart, skeletal muscle with calcification
7	emu	3 weeks	F	heart, neck muscles
8	emu	5 weeks	F	heart, skeletal muscle
9	emu	6 weeks	M	heart, skeletal muscle, femur fracture

TABLE 5

## Selenium &amp; Vitamin E in Cases 1-9

Case	liver selenium	liver vitamin E	dietary selenium	dietary vitamin E
1	ND	ND	ND	ND
2	0.495 ppm	ND	ND	ND
3	ND	ND	ND	ND
4	ND	ND	ND	ND
5	ND	ND	ND	ND
6	0.175 ppm	0.69 µg/g	ND	ND
7	0.822 ppm	9.10 µg/g	ND	ND
8*	0.986 ppm	2.04 µg/g	0.270 ppm	12.02 µg/g
9*	3.737 ppm	31.08 µg/g	injected	injected

ND = not determined

\*Cases 8 and 9 were from the same farm (Emu Farm #7). Case 8 died prior to treatment with vitamin E and Case 9 was euthanized due to femur fracture after receiving injections of selenium and vitamin E (selenium at 0.06 mg/kg BW and vitamin E at 3.0 mg/kg BW) intramuscularly for three consecutive days.

observed in skeletal and cardiac muscle. The liver selenium level was 0.986 ppm wet weight. The liver vitamin E level was 2.05  $\mu\text{g/g}$  of tissue.

The emu chick in Case 9 (from the same farm as Case 8) subsequently became depressed and refused to rise. The owner treated this chick with 250 mg ampicillin orally, trimethoprim/sulfamethoxazole (240 mg/ml) intramuscularly at the rate of 1.0 milliliter per pound of body weight twice daily because Group E *Salmonella* and *Erysipelothrix rhusiopathiae* had been isolated from birds on the premises. In addition, an injectable selenium and vitamin E preparation (Bo-Se; Burns-Biotec) was administered: selenium at the rate of 0.06 mg/kg of body weight and vitamin E at the rate of 3.0 mg/kg body weight for three consecutive days. The chick rallied and appeared much improved clinically, but was found with a fractured femur the fourth day after initiation of therapy. Despite therapy, histologic lesions of degenerative myopathy were observed in this chick, as well. The liver selenium level was 3.738 ppm wet weight. The toxic liver selenium levels in poultry are considered to be above 4.00 ppm wet weight. This illustrates how quickly toxic levels of selenium can accumulate during treatment. The liver vitamin E level was 31.08  $\mu\text{g/g}$  of tissue. The pelleted ration was found to contain 0.270 ppm of selenium and 12.02  $\mu\text{g}$  of vitamin E/g of feed (equivalent to 13 IU of vitamin E/kg of feed).

Both Cases 8 and 9 came Emu Farm #7, which received a farm visit. Blood was collected from a group of 5 emu chicks, aged 5 to 9 weeks old. The blood selenium and plasma vitamin E data from Emu Farm #7 is presented in Table 6. The mean blood selenium for this group was 0.536 ppm (range 0.421 - 0.654 ppm) and

the mean plasma vitamin E was 0.88  $\mu\text{g/ml}$  (range 0.70 - 1.04  $\mu\text{g/ml}$ ). The dietary selenium level was 0.270 ppm and the dietary vitamin E level was 12.02  $\mu\text{g/g}$  of feed (equivalent to 13 IU/kg of feed). Each chick in the group received a single intramuscular injection of vitamin E (Rocavit E; Roche) at the rate of 5.0 mg of vitamin E/kg of body weight. The group was then maintained on their normal pelleted ration, supplemented with a granular vitamin E feed supplement (Vitamin E '20' (20,000 IU/lb); Leo Cook Company) at the rate of 100-200 IU/kg of feed; no additional selenium was added to the existing ration.

No additional morbidity or mortality occurred on this farm in the following four weeks. The farm was revisited four weeks after the initial visit, having been maintained on the supplemented feed. Only one bird was still available for sampling because the owner had sold all the other birds. This bird's blood selenium was 0.704 ppm and the plasma vitamin E was 2.52  $\mu\text{g/ml}$ .

TABLE 6

Vitamin E and Selenium Data from Emu Farm #7

Bird (emu)	Blood selenium ppm	Plasma Vitamin E $\mu\text{g/ml}$	Dietary selenium ppm	Dietary Vitamin E $\mu\text{g/g}$
E7-1 (untreated)	0.575	0.70	0.270	12.02
E7-2 (untreated)	0.528	0.85	0.270	12.02
E7-3 (untreated)	0.654	1.04	0.270	12.02
E7-4 (untreated)	0.503	1.01	0.270	12.02
E7-5 (untreated)	0.421	0.82	0.270	12.02
Mean	$0.536 \pm .086$	$0.88 \pm 0.14$	0.270	12.02
E7-4 (treated)	0.704	2.52	0.270	100-200

### **Description of the Lesions of Degenerative Myopathy**

Lesions variably involved skeletal muscle, myocardium and gizzard smooth muscle. Muscles of the hindlimbs, usually the adductors, muscles of the lumbar region and the muscles of the dorsal cervical region were the muscle groups most commonly affected. Often gross lesions were very subtle. Degenerating muscle may be very difficult to detect grossly when it is uncalcified. There may be paleness and possibly white streaks in the muscles. Histologic examination is necessary to confirm the presence of degenerative myopathy.

The histologic lesions ranged from areas of acute necrosis to scattered individual degenerating myocytes, depending on the region examined. The whole gamut of degeneration was seen. The myopathy was characterized by loss of striations, hyalinization, necrosis and segmental fragmentation of myofibers with proliferation of satellite nuclei and invasion by macrophages. Retraction caps and discoid degeneration were commonly observed. Calcification was seen in some cases and was particularly obvious in the rhea in Case 6, but it was not consistently present in all cases. Eventual fibrosis would be expected in chronic healing lesions, but this phenomena was not observed in this group of cases.

### **Results of the Survey of Circulating Blood Selenium and Plasma Vitamin E in Ostriches and Emus**

The blood selenium, plasma vitamin E, dietary selenium and dietary vitamin E determinations from a total of 75 ostriches from 9 different farms and a total of 62

emus from 6 different farms are presented in Tables 7 and 8. Tables 7 and 8 present the mean blood selenium and plasma vitamin E values for each farm with its corresponding standard deviation of the farm mean. Each farm had a single dietary determination of selenium and vitamin E.

TABLE 7  
Ostrich Survey Data

Ostrich Group	n	Mean blood selenium (ppm)	Mean plasma vitamin E ( $\mu\text{g/ml}$ )	Dietary selenium (ppm)	Dietary vitamin E ( $\mu\text{g/g}$ )
1 - EC	5	.670 $\pm$ .049	1.68 $\pm$ .59	0.537	93.53
2 - ST	10	.557 $\pm$ .113	2.34 $\pm$ .67	0.511	190.72
3 - GR	4	.691 $\pm$ .055	1.60 $\pm$ .81	0.746	143.27
4 - BO	2	.770 $\pm$ .049	1.25 $\pm$ .21	0.347	178.07
5 - KE	6	.724 $\pm$ .079	1.67 $\pm$ .39	0.388	136.14
6 - JO	9	.677 $\pm$ .127	1.30 $\pm$ .21	0.522	11.94
7 - JSA	22	.589 $\pm$ .070	.97 $\pm$ .35	0.481	53.01
8 - JSB	11	.525 $\pm$ .032	.81 $\pm$ .50	0.358	32.22
9 - WS	6	.563 $\pm$ .046	.96 $\pm$ .48	ND	14.43
Total	75				

TABLE 8  
Emu Survey Data

Emu Group	n	Mean blood selenium (ppm)	Mean plasma vitamin E ( $\mu\text{g/ml}$ )	Dietary selenium (ppm)	Dietary vitamin E ( $\mu\text{g/g}$ )
1 - HY	2	.841 $\pm$ .061	2.96 $\pm$ 0.47	1.315	100.01
2 - PA	5	.830 $\pm$ .056	4.45 $\pm$ 2.13	1.214	101.19
3 - TE	3	.826 $\pm$ .061	3.32 $\pm$ 0.99	1.315	100.01
4 - ST	6	.787 $\pm$ .023	2.67 $\pm$ 0.48	0.737	119.77
5 - CO	41	.696 $\pm$ .102	1.03 $\pm$ 0.45	0.454	37.29
6 - HU	5	.623 $\pm$ .181	3.10 $\pm$ 1.14	ND	182.47
Total	62				

## DISCUSSION

Demonstration of vitamin E or selenium deficiency as a cause of the degenerative myopathy described herein was hampered by the lack of information on the normal and deficient levels of selenium and vitamin E in blood and tissues of ratite species. The history was very important in ruling out or narrowing down the other possible causes of degenerative myopathy in birds. Response to treatment was extremely important in distinguishing whether a deficiency of vitamin E was the primary cause of this myopathy.

Deficient, marginally deficient, adequate and toxic levels of selenium in blood, tissue and diet have been well characterized in poultry, but not in ratites. Extrapolation from poultry may not be appropriate, but it appears to be the best data available at this time. Judging from the selenium research in poultry, all the ratite diets examined in this study were within the adequate range (0.30 to 1.10 ppm dry weight). Liver selenium levels in poultry very closely correlate with dietary selenium: adequate liver selenium is 0.35 - 1.00 ppm wet weight, while adequate dietary selenium is 0.30 to 1.10 ppm dry weight. Using the poultry levels for liver, only the rhea in Case 6 had a liver selenium level in the deficient range. It is suspected that this rhea (Case 6) also had a low liver vitamin E level.

Selenium toxicity is a very real hazard in oversupplementing ratites, either with injectable formulations or in the diet. Note that the liver selenium (3.737 ppm) in the emu in Case 9 that was treated for 3 consecutive days with the recommended

dose of injectable vitamin E/selenium, closely approached the toxic range described for poultry. It appears that this method of supplementation, although considered empirically appropriate without prior knowledge of blood, tissue or dietary selenium levels, needs to be utilized cautiously, since toxic levels of selenium can be reached rapidly. For this reason, and the reason that the therapeutic range of selenium is so narrow, initial injectable vitamin E alone followed by dietary supplementation of vitamin E alone was implemented on Emu Farm #7.

Plasma vitamin E, measured as  $\alpha$ -tocopherol, has been shown to correlate well (correlation coefficient  $r > .90$ ) with dietary levels in poultry<sup>109</sup> and several mammalian species.<sup>110</sup> The typical poultry diet contains 10-30  $\mu\text{g/g}$  (10-30 IU/kg) of diet. Turkeys raised for slaughter had plasma vitamin E levels of  $2.4 \pm 1.0$   $\mu\text{g/ml}$ .<sup>111</sup> Six-week-old turkey poults fed vitamin E-deficient diets containing 2-3 IU vitamin E/kg of diet had plasma vitamin E levels averaging  $0.23 \pm 0.03$   $\mu\text{g/ml}$ , while poults fed diets containing no detectable vitamin E had significantly ( $p < 0.005$ ) lower plasma concentrations of  $0.10 \pm 0.04$   $\mu\text{g/ml}$  and displayed encephalomalacia.<sup>112</sup> This information strongly suggests that plasma vitamin E is a good indicator of dietary vitamin E consumption. However, the length of time a specific diet is ingested also appears to be a significant factor as well. A study using peregrine falcons (*Falco peregrinus*) experiencing increased chick mortality, showed that significant increases in circulating levels occurred after feeding a given level of vitamin E for several months.<sup>91</sup>

Problems that would impair the digestion or absorption of lipids can be expected to result in lowered plasma vitamin E concentrations. This has been described in chickens with nutritional pancreatic atrophy, in which the primary selenium deficiency results in the pancreatic atrophy, but the subsequent impairment in fat absorption results in a secondary vitamin E deficiency, despite adequate dietary levels of vitamin E.

Previous studies in poultry have also shown that plasma vitamin E correlates well with tissue levels in heart, liver and lung (correlation coefficients .910, .925 and .971, respectively).<sup>109</sup> In chicks fed diets supplemented with vitamin E (0 - 500 IU/kg diet), liver levels increased linearly from 10 to > 100  $\mu\text{g/g}$  of liver wet weight.<sup>31</sup> This information strongly suggests that tissue vitamin E (heart, liver or lung) can be used as a measure of plasma vitamin E. Studies in poultry have also shown that vitamin E does not reach saturation in tissue with dietary vitamin E contents up to at least 180  $\mu\text{g/g}$  (or approximately 180 IU/kg of feed).<sup>109</sup>

In the present study, there appears to be a marked increased incidence of degenerative myopathy in juvenile birds. Ratite chicks are extremely fast-growing during their initial 8 to 10 months. The increased incidence may be related to this fast growth. It has been speculated that in poultry higher levels of vitamin E are needed when muscle protein turnover and amino acid requirements are greatest, i.e., during periods of rapid skeletal growth.<sup>13</sup> This may explain the higher incidence of degenerative myopathy in juvenile ratites.

Comparisons of differences between blood selenium and plasma vitamin E values between the farms could not be statistically tested because the values obtained from each farm were not independent observations due to fact that all the birds on each farm were on the same diet. This problem could be remedied in future studies by blocking in the experimental design of a more controlled study.

Meaningful, statistically significant comparisons between the plasma vitamin E levels and the dietary vitamin E level, and likewise between the blood selenium levels and the dietary selenium levels, were explored, but several problems were encountered. First, only the samples of pelleted feed offered to the birds were analyzed. In many situations, ratites are also allowed to graze on various grasses or may be supplemented with various hays or forages. These pastures and forages can be expected to contain variable amounts of vitamin E and selenium and also different forms of vitamin E and selenium that would differ in their bioavailability. Putting all of these confounding variables together could significantly affect the plasma vitamin E and blood selenium values to an unacceptable degree of uncertainty. Therefore, no statistical analysis is presented on these comparisons. Again, a controlled experiment with birds assigned to a given diet, excluding all other feedstuffs and providing a common, known form of selenium and vitamin E with known bioavailability, would remove these confounding variables and provide more meaningful statistical comparisons. The high cost of the experimental units in such a controlled experiment was beyond the scope of the current study.

The current study is an observational one which was considered exploratory in nature, attempting to determine the range of dietary constituents fed to ratites. The information from this study could serve as a basis for designing a more controlled experiment.

The diets in this survey could not be compared statistically between the farms because the sample size of one diet per farm caused the comparison to be untestable. In general, however, it can be seen from the data table that the lower the dietary selenium or vitamin E, the lower the blood selenium and plasma vitamin E.

The mean of the farm means were used in the following comparisons because, statistically, that mean would be expected to be a better estimate of the true mean of the blood selenium and plasma vitamin E values for each species. Using Levene's test, it was determined that the variance between the farm means was not great enough to cause an error in rejection of a hypothesis involving these means.

There is little information on the normal plasma or serum vitamin E levels in ratites. Jensen and Hicks did look at serum vitamin E levels in clinically normal, adult ratites: 23 ostriches and 23 emus. Their mean serum vitamin E level was 2.1  $\mu\text{g/ml}$  in ostriches and 2.39  $\mu\text{g/ml}$  in emus.<sup>113</sup> The only plasma vitamin E values found in the literature from healthy rheas (*Rhea americana*) were a mean of  $11.60 \pm 1.11$   $\mu\text{g/ml}$  (n=5)<sup>83</sup> and a mean of 8.9  $\mu\text{g/ml}$  (n=7)<sup>114</sup> The mean plasma vitamin E values for two ostriches were reported as 6.0  $\mu\text{g/ml}$  (n=2).<sup>114</sup> In general, plasma vitamin levels are higher in carnivorous birds than in herbivorous birds.<sup>114</sup>

The results from the present survey of plasma vitamin E were compared to the study done by Jensen and Hicks. The mean of the farm means ( $1.41 \pm 0.48 \mu\text{g/ml}$  for ostriches and  $2.92 \pm 1.11 \mu\text{g/ml}$  for emus) was used as a better estimate of the true mean. Using the Student's one-sample t-test, the mean ostrich plasma vitamin E level found by Jensen and Hicks was significantly different from the mean plasma vitamin E levels found in the current study ( $p = .003$ ; 2-sided). The mean emu plasma vitamin E level found by Jensen and Hicks was not significantly different from the mean plasma vitamin E level found in the current study ( $p = .294$ ; 2-sided).

Explanation of the significant difference between ostrich circulating vitamin E levels in the Jensen and Hicks study and the current study may lie in the fact that the ostriches in the Jensen and Hicks study were adults and the ostriches in the current study were a mixture of adults and juveniles. Another, perhaps more prominent, reason for the difference may lie in the possibility that dietary levels of vitamin E may have been consistently higher in the Jensen and Hicks study than the dietary levels in the current study.

Results from the pre-treatment group at Emu Farm #7 were compared to the results of the survey of normal emus. The Emu Farm #7 mean plasma vitamin E values were found to be significantly different from the mean of the means from emu farms 1 through 6 ( $p = .003$ ; 2-sided; Student's t-test). The Emu Farm #7 mean blood selenium values were also found to be significantly different from the mean of the means from emu farms 1 through 6 ( $p\text{-value} = .002$ ; 2-sided). Therefore, the chicks from Emu Farm #7, which was experiencing degenerative myopathy in its

population, did have lower than normal blood selenium and plasma vitamin E levels when compared to normal, unaffected emus.

Only one chick remained at Emu Farm #7 when post-treatment sampling was done four weeks later. This single chick's blood selenium was 0.704 ppm and the plasma vitamin E was 2.52 µg/ml. The only change made on this farm was a single injection of vitamin E (5.0 mg/kg body weight) per bird and the addition of 100-200 IU of vitamin E/kg of feed. Although statistical significance between the pre-treatment and post-treatment groups could not be tested statistically because of the sample size of one in the post-treatment group, the marked increase in plasma vitamin E in this chick and the report from the farm owner that morbidity and mortality had ceased are good evidence that the degenerative myopathy in this flock was at the very least vitamin E- responsive, if not outright vitamin E deficiency disease.

## SUMMARY

This paper describes the gross and histologic lesions of degenerative myopathy in several ratites and details the response to vitamin E supplementation on one emu farm. It also presents the results of an observational survey of blood selenium and plasma vitamin E levels in two ratite species, as well as the dietary levels of those nutrients that produced those blood levels. The data suggests that plasma vitamin E and whole blood selenium can be used as a rough measure of the adequacy of these nutrients in the diet. Additional controlled studies are needed to fine-tune these rough estimates.

When the case study information in this thesis was initially presented,<sup>115</sup> ratite breeders in Oregon were feeding rations prepared by local feed mills that were based on the requirements for poultry. Therefore, many of the rations produced in Oregon contained the recommended levels of dietary vitamin E for poultry (10-30 IU/kg feed). Oregon is known to be an area of the country that has very selenium deficient soils, so the local feed producers often increased the feed selenium level to compensate for this. This explains why the feed selenium and blood selenium levels appear adequate in most situations.

During the time following the completion of this study, nationally recognized ratite feed producers have been producing diets that contain between 160 and 300 IU of vitamin E/kg of feed.<sup>116</sup> This probably exceeds the true dietary requirement for growing ratite chicks, but degenerative myopathy has not been seen at these dietary levels.

As the ratite industry moves toward a slaughter market and marginal profit decreases, it will be necessary to more closely define the minimum and optimal dietary vitamin E and selenium levels needed for cost-effective ratite production. In the past, selenium and vitamin E were considered interchangeable. There is a growing body of knowledge that suggests, especially in exotic or zoo species, that these two nutrients are not completely interchangeable,<sup>83,93,114</sup> and that vitamin E supplementation is often necessary. The optimal dietary levels of each nutrient, selenium and vitamin E, will need to be determined, as well as their nutrient interactions in these various species.

Given the problems seen on Emu Farm #7, the levels of vitamin E that are required for poultry do not appear to be adequate for juvenile emus, and probably other ratites, as well. Researchers in zoos suggest that exotic species may require as much as 10 times the recommended intake of vitamin E to prevent deficiency diseases.<sup>83,93,114</sup> Ten times the recommended poultry level of dietary vitamin E would be 100-300 IU/kg of feed. This may be overkill at the 300 IU/kg level, but vitamin E levels in the range of 100-200 IU/kg of feed appear to be more appropriate than the 30 IU/kg level for the prevention of vitamin E-responsive degenerative myopathy in ratites. Since vitamin E levels in feed decline over time and high PUFAs in the diet tend to increase the need for vitamin E, addition of vitamin E at 300 IU/kg of feed to compensate for these deteriorations may be appropriate in prevention of vitamin E deficiency diseases.

## BIBLIOGRAPHY

1. Stewart JS. Ratites. In: Ritchie BW, Harrison GJ, Harrison L, eds. *Avian medicine: principles and applications*. Lake Worth, Florida: Wingers Publishing, 1994:1284-1326.
2. Jenkins J. Ratite medicine and surgery. In: Roskopf WJ, Woerpel RW, eds. *Diseases of cage and aviary birds*. 3<sup>rd</sup> ed. Philadelphia: Williams & Wilkins, 1996:1002-1006.
3. Fowler M. Clinical anatomy of ratites. In: Tully TN, Shane SM, eds. *Ratite management, medicine & surgery*. Malabar, Florida: Krieger Publishing Company, 1996:1-10.
4. Bruning DF, Dolensek EP. Ratites. In: Fowler ME, ed. *Zoo and wild animal medicine*. 2<sup>nd</sup> ed. Philadelphia: WB Saunders Co., 1986:277-291.
5. Combs GF Jr, Combs S. Biochemical Functions of Selenium, Chapter 6. In: *The role of selenium in nutrition*. New York: Academic Press, 1986:206-265.
6. Arthur JR, Nicol F, Beckett GJ. Hepatic iodothyronine-5'-deiodinase: the role of selenium. *Biochem J* 1990;272:537-546.
7. Behne D, Kyriakopoulos A, Meinhjold H, Kohrle J. Identification of type 1 iodothyronine-5'-deiodinase as a selenoenzyme. *Biochem Biophys Res Commun* 1990; 173(3):1143-1152.
8. Vanderpas JB, Contempre B, Duale NL, Goossens W, et al. Iodine and selenium deficiency associated with cretinism in northern Zaire. *Am J Clin Nutr* 1990;52:1087-1093.
9. Vanderpas JB, Contempre B, Duale NL, Deckx H, et al. Selenium deficiency mitigates hypothyroxinemia in iodine-deficient subjects. *Am J Clin Nutr* 1993;57 (suppl): 271S-275S.
10. Century B, Hurwitt ML. Effect of dietary selenium on incidence of nutritional encephalomalacia in chicks. *Proc Soc Exp Biol Med* 1964;117:320-322.
11. Combs GF Jr, Hady MM. Selenium involved with vitamin E in preventing encephalomalacia in the chick. *FASEB J* 1991;5:A581.

12. Mercurio SD, Combs GF Jr. Synthetic seleno-organic compound with glutathione peroxidase-like activity in the chick. *Biochem Pharmacol* 1986;35:4505-4509.
13. Combs GF Jr. Clinical implications of selenium and vitamin E in poultry nutrition. *Vet Clin Nutr* 1994;1(3):133-140.
14. Machlin LJ, Shalkop WT. Muscular degeneration in chickens fed diets low in vitamin E and sulfur. *J Nutr* 1956;60:87-96.
15. Calvert CC, Scott ML. Effect of selenium on the requirement for vitamin E and cystine for the prevention of nutritional muscular dystrophy in the chick. *Fed Proc* 1963;22:318-319.
16. Hull SJ, Scott ML. A study of the relationship of plasma glutamic-oxaloacetic transaminase activity to nutritional muscular dystrophy in the chick. *J Nutr* 1972;102:1367-1376.
17. Shih JCH, Jonas RH, Scott, ML. Oxidative deterioration of the muscle proteins during nutritional muscular dystrophy in chicks. *J Nutr* 1977;107:1786-1791.
18. Hathcock J. Sulfur metabolism in nutritional muscular dystrophy in the chicken. PhD Thesis, Cornell University, Ithaca, NY, 1967.
19. Thompson JN, Scott ML. Role of selenium in the nutrition of the chick. *J Nutr* 1969;97:335-342.
20. Thompson JN, Scott ML. Impaired lipid and vitamin E absorption related to atrophy of the pancreas in selenium-deficient chicks. *J Nutr* 1970;100:797-809.
21. Gries CL, Scott ML. Pathology of selenium deficiency in the chick. *J Nutr* 1972;102:1287-1296.
22. Combs GF Jr, Bunk MJ. The role of selenium in pancreatic function. In: Spallholz JE, Martin, JL, Ganther HE, eds. *Selenium in biology and medicine*. Westport, CT: Avi Publishing Company, 1981:70.
23. Whitacre ME, Combs GF Jr. Selenium and mitochondrial integrity in the pancreas of the chick. *J Nutr* 1983;113:1972-1983.
24. Bunk MJ, Combs GF Jr. Relationship of selenium-dependent glutathione peroxidase activity and nutritional pancreatic atrophy in selenium-deficient chicks. *J Nutr* 1981;111:1611-1620.

25. Root EJ, Combs GF Jr. Disruption of endoplasmic reticulum is the primary ultrastructural lesion of the pancreas in the selenium-deficient chick. *Proc Soc Exp Biol Med* 1988;187:513-521.
26. Bunk MJ, Combs GF Jr. Effect of selenium on appetite in the selenium-deficient chick. *J Nutr* 1980;110:743-749.
27. Bunk MJ, Combs GF Jr. Evidence for an impairment in the conversion of methionine to cysteine in the selenium-deficient chick. *Proc Soc Exp Biol Med* 1981;167:87-93.
28. Halpin KM, Baker DH. Selenium deficiency and transsulfuration in the chick. *J Nutr* 1984;114:606-612
29. LaVorgna MW, Combs GF Jr. Evidence of a hereditary factor affecting the chick's response to uncomplicated selenium deficiency. *Poultry Sci* 1983;62:164-168.
30. Combs GF Jr, Liu CH, Lu ZH, Su Q. Uncomplicated selenium deficiency produced in chicks fed a corn-soy-based diet. *J Nutr* 1984;114:964-976.
31. Whitacre ME, Combs GF Jr, Combs SB, Parker RS. Influence of dietary vitamin E deficiencies in nutritional pancreatic atrophy in selenium-deficient chicks. *J Nutr* 1987;117:460-467.
32. Marsh JA, Dietert RR, Combs GF Jr. Influence of dietary selenium and vitamin E on the humoral immune response of the chick. *Proc Soc Exp Biol Med* 1981;166:228-236.
33. Cantor AH, Scott ML. The effect of selenium in the hen's diet on egg production, hatchability, performance of progeny and selenium concentration in eggs. *Poultry Sci* 1974;53:1870-1880.
34. Latshaw JD, Ort JF, Diesem CD. The selenium requirements of the hen and effects of a deficiency. *Poultry Sci* 1977;56:1876-1881
35. Combs GF Jr, Scott M. The selenium needs of laying and breeding hens. *Poultry Sci* 1979;58:871-884.
36. Walter WC, Jensen LS. Effectiveness of selenium and non-effectiveness of sulfur amino acids in preventing muscular dystrophy in the turkey poult. *J Nutr* 1963;80:327-331.

37. Cantor AH, Moorhead PD, Musser MA. Comparative effects of selenite and seleno-methionine upon nutritional muscular dystrophy, selenium-dependent glutathione peroxidase, and tissue selenium concentrations of turkey poults. *Poultry Sci* 1982;61:478-484.
38. Walter ED, Jensen JS. Serum glutamic-oxaloacetic transaminase levels, muscular dystrophy and certain hematological measurements in chicks and poults as influenced by vitamin E, selenium and methionine. *Poultry Sci* 1964;43:919-926.
39. Scott ML, Olson G, Krook L, Brown WR. Selenium-responsive myopathies of myocardium and of smooth muscle in the young poult. *J Nutr* 1967;91:573-583.
40. Harper JA, Helfer DH. The effect of vitamin E, methionine and selenium on degenerative myopathy of turkeys. *Poultry Sci* 1972;51:1757-1759.
41. Van Vleet JF, Farrans VJ. Ultrastructural alterations in gizzard smooth muscle of selenium-vitamin E-deficient ducklings. *Avian Dis* 1977;21:531-542.
42. Van Vleet JF, Farrans VJ. Ultrastructural alteration in skeletal muscle of ducklings fed a selenium-vitamin E-deficient diet. *Am J Vet Res* 1977;38:1399-1405.
43. Dean WF, Combs GF Jr. Influence of dietary selenium in performance, tissue selenium content, and plasma concentration of selenium-dependent glutathione peroxidase, vitamin E and ascorbic acid in ducklings. *Poultry Sci* 1980;60:2655-2663.
44. Brown RG, Sweeny PR, Moray ET Jr. Collagen levels in tissues from selenium-deficient ducks. *Comp Biochem Physiol* 1982;72A:383-389.
45. Bartlett MW, Egelstaff PA, Holden TM, Stinson RH, et al. Structural changes in tendon collagen resulting from muscular dystrophy. *Biochem Biophys Acta* 1973;328:213-220.
46. Brown RG, Sweeney PR, George JC, Stanley DW, et al. Selenium deficiency in the duck: serum ascorbic acid levels in developing muscular dystrophy. *Poultry Sci* 1974;53:1235-1239.
47. Moran ET Jr, Carlson HC, Brown RG, Sweeney PR, et al. Alleviating mortality associated with a vitamin E-selenium deficiency by dietary ascorbic acid. *Poultry Sci* 1975;54:266-269.
48. Combs GF Jr, Pesti GM. Influence of ascorbic acid on selenium nutrition in the chick. *J Nutr* 1976;106:958-966.

49. Jager FC. Effect of dietary linoleic acid and selenium on the requirement of vitamin E in ducklings. *Nutr Metab* 1977;14:210-227.
50. Scott ML, Thompson JN. Selenium in nutrition and metabolism. *Proc Maryland Nutr Conf* 1968;1.
51. Jensen LS. Selenium deficiency and impaired reproduction in Japanese quail. *Proc Soc Exp Biol Med* 1968;128:970-972.
52. Van Heerden J, Hayes SC, Williams MC. Suspected vitamin E — selenium deficiency in two ostriches. *J South African Vet Assoc* 1983;54(1):53-54.
53. Vorster BJ. Nutritional muscular dystrophy in a clutch of ostriches. *J South African Vet Assoc* 1984;55(1):39-40.
54. Philbey AW, Button C, Gestier AW, et al. Anasarca and myopathy in ostrich chicks. *Aust Vet J* 1991;68(7):237-230.
55. Kinder LL, Angel CR, Anthony NB. Apparent selenium toxicity in emus (*Dromaius novaehollandiae*). *Avian Dis* 1995;39:652-657.
56. National Research Council. *Nutrient requirements for poultry*. 9<sup>th</sup> ed. Washington, D.C.: National Academy of Sciences Press, 1994.
57. Emsminger ME, Oldfield JE, Heinemann WW. *Feeds & nutrition*. 2<sup>nd</sup> ed. Clovis, California: Ensminger Publishing Co, 1990:232.
58. Ohlendorf HM. Selenium. In: Fairbrother A, Locke LH, Hoff GL, eds. *Noninfectious diseases of wildlife*. 2<sup>nd</sup> ed. Ames, Iowa: Iowa State University Press, 1996;128-140.
59. Puls R. *Veterinary trace mineral deficiency and toxicity information, publication 5139*. Abbotsford, B.C., Canada: Provincial Veterinary Diagnostic Laboratory, 1981;194.
60. National Research Council. Vitamin E. In: *Vitamin Tolerance of Animals*. Washington, D.C.: National Academy of Sciences Press, 1987:23-30.
61. Calle PP, Dierenfeld ES, Robert ME. Serum  $\alpha$ -tocopherol in raptors fed vitamin E-supplemented diets. *J Zoo & Wildl Med* 1989;20(1):62-67.

62. Nichols DK, Wolff MJ, Phillips LG, Montali RJ. Coagulopathy in pink-backed pelicans (*Pelecanus rufescens*) associated with hypervitaminosis E. *J Zoo & Wildl Med* 1989;20(1):57-61.
63. Corrigan JJ, Marcus FI. Coagulopathy associated with vitamin E ingestion. *J Am Med Assoc* 1974;230:1300-1301.
64. March BE, Wong E, Seier L, et al. Hypervitaminosis E in the chick. *J Nutr* 1973;103:371-377.
65. Bettger WJ, Olson RE. Effect of alpha-tocopherol and alpha-tocopherolquinone on vitamin K-dependent carboxylation in the rat. *Fed Proc* 1982;41:344
66. Olson RE, Jones JP. The inhibition of vitamin K action by D-alpha-tocopherol and its derivatives. *Fed Proc* 1979;38:710.
67. Rao GH, Mason KE. Antisterility and antivitamin K activity of D-alpha-tocopherol hydroquinone in the vitamin E-deficient female rat. *J Nutr* 1975;105:495-498.
68. Tully TN, Hodgkin C, Morris JM, et al. Exertional myopathy in an emu (*Dromaius novaehollandiae*). *J Avian Med & Surgery* 1996;10(2):96-100.
69. Carpenter JW, Thomas NJ, Reeves S. Capture myopathy in an endangered sandhill crane (*Grus canadensis pulla*). *J Zoo Wildl Med* 1991;22(4):488-493.
70. Brannian RE, Graham DL, Creswell J. Restraint associated myopathy in East African crowned cranes, in *Proceedings*. American Association of Zoo Veterinarians 1981;21-23.
71. Wingingstad RM, Hurley SS, Sileo L. Capture myopathy in a free-flying greater sandhill crane (*Grus canadensis tabida*) from Wisconsin. *J Wild Dis* 1983;19:289-290.
72. Heldstab A, Ruedi D. The occurrence of myodystrophy in zoo animals at the Basle Zoological Garden. In: Montali RJ, Migaki G, eds. *The comparative pathology of zoo animals*. Washington, D.C.: Smithsonian Institute Press, 1980:27-34.
73. Young E. Leg paralysis in the greater flamingo and lesser flamingo (*Phoenicopterus ruber roseus* and *Phoeniconaias minor*) following capture and transportation. *Int Zoo Yearb* 1967;7:226-227.

74. Wobeser GA. *Diseases of wild waterfowl*. New York: Plenum Press, 1981.
75. Wobeser GA, Howard J. Mortality of waterfowl on a hypersaline wetland as a result of salt encrustation. *J Wild Dis* 1987;12:127-134.
76. Bollinger T, Wobeser GA, Clark RG, et al. Concentration of creatine kinase and aspartate aminotransferase in the blood of wild mallards following capture by three methods for banding. *J Wildl Dis* 1989;25:225-231.
77. Dabbert CB, Powell KC. Serum enzymes as indicators of capture myopathy in mallards (*Anas platyrhynchos*). *J Wild Dis* 1993;29:304-309.
78. Spraker TR, Adrian WR, Lance WR. Capture myopathy in wild turkeys (*Meleagris gallopavo*) following trapping, handling, and transportation in Colorado. *J Wildl Dis* 1987;23:447-453.
79. Williams ES, Thorne ET. Exertional myopathy (capture myopathy). In: Fairbrother A, Locke LH, Hoff GL, eds. *Noninfectious diseases of wildlife*. 2<sup>nd</sup> ed. Ames, Iowa: Iowa State University Press, 1996;181-193.
80. Hulland TJ. Muscle and tendon. In: Jubb KVF, Kennedy PC, Palmer N, eds. *Pathology of domestic animals*. Vol. 1. 4<sup>th</sup> ed. New York: Academic Press, 1993;183-265.
81. Mann FA, Helmick KE. Shock. In: Fairbrother A, Locke LH, Hoff GL, eds. *Noninfectious diseases of wildlife*. 2<sup>nd</sup> ed. Ames, Iowa: Iowa State University Press, 1996;173-180.
82. Chalmers GA, Barrett MW. Capture myopathy. In: Hoff GL, Davis JW, eds. *Noninfectious disease of wildlife*. Ames, Iowa: Iowa State University Press, 1982;84-94.
83. Dierenfeld ES. Vitamin E deficiency in zoo reptiles, birds and ungulates. *J Zoo & Wildl Med* 1989;20(1):3-11.
84. Carpenter JW, Spann JW, Novilla MN. Diet-related die-off of captive black-crowned night herons, in *Proceedings*. Annu Conf Am Assoc Zoo Vet 1979;51-55.
85. Geraci JR, St. Aubin DJ. Nutritional disorders of captive fish-eating animals. In: Montali RJ, Migaki G, eds. *The comparative pathology of zoo animals*. Washington, D.C.: Smithsonian Institute Press, 1979;41-49.

86. Lowenstine LJ. Nutritional disorders of birds. In: Fowler ME, ed. *Zoo and wild animal medicine*. 2<sup>nd</sup> ed. Philadelphia: WB Saunders Co, 1986;201-212.
87. Nichols DK, Campbell VL, Montali RJ. Pansteatitis in great blue herons. *J Am Vet Med Assoc* 1986;189(9):1110-1112.
88. Nichols DK, Montali RJ. Vitamin E deficiency in captive and wild piscivorous birds, in *Proceedings*. 1<sup>st</sup> Internat Conf Zool Avian Med 1987;419-421.
89. Wagner JE, Dietlein DR. Steatitis in an American white pelican. *Internat Zoo Yearb*, 1970;10:174.
90. Campbell G, Montali RJ. Myodegeneration in captive brown pelicans attributed to vitamin E deficiency. *J Zoo Animal Med* 1980;11:35-40.
91. Dierenfeld ES, Sandfort CE, Satterfield WC. Influence of diet on plasma vitamin E in captive peregrine falcons. *J Wildl Manag* 1989;53(1):160-164.
92. Graham DL, Halliwell WH. Malnutrition in birds of prey. In: Fowler ME, ed. *Zoo and wildlife medicine*. 2<sup>nd</sup> ed. Philadelphia: WB Saunders Co, 1986;379-385.
93. Calle PP, Dierenfeld ES, Robert ME. Serum  $\alpha$ -tocopherol in raptors fed vitamin E-supplemented diets. *J Zoo & Wildl Med* 1989;20(1):62-67.
94. Liu SK, Dolensek EP, Tappe JP. Cardiomyopathy and vitamin E deficiency in zoo animals and birds. *Heart & Vessels* 1985; Suppl 1:228-293.
95. Campbell TW. Hypovitaminosis E: its effect on birds, in *Proceedings*. 1<sup>st</sup> Internat Conf Zool Avian Med 1987;75-77.
96. Dhillon AS, Winterfield RW. Selenium-vitamin E deficiency in captive wild ducks. *Avian Dis* 1983;27(2):527-530.
97. Roffe TJ, Stroud RK, Windingstad RM. Suspected fusariomycotoxicosis in sandhill cranes (*Grus canadensis*): clinical and pathological findings. *Avian Dis* 1989;33:451-457.
98. Windingstad RM, Cole RJ, Nelson PE, et al. *Fusarium* mycotoxins from peanuts suspected as a cause of sandhill crane mortality. *J Wildl Dis* 1989;25(1):38-46.
99. Julian RJ, Brown TP. Poisons and toxins. In: Calnek BW, ed. *Diseases of Poultry*. 10<sup>th</sup> ed. Ames, Iowa: Iowa State University Press, 1997;979-1005.

100. Simpson CR, Damron BL, Harms RH. Toxic myopathy of chicks feed *Cassia occidentalis*. *Avian Dis* 1971;15:284-290
101. Wilson BW. Developmental and maturational aspects of inherited avian myopathies. *Proc Soc Experimental Biol Med* 1990;194(2);87-96.
102. Riddell C. Developmental, metabolic, and other noninfectious disorders. In: Calnek BW, ed. *Diseases of Poultry*. 10<sup>th</sup> ed. Ames, Iowa: Iowa State University Press, 1997; 913-950.
103. Matsui T, Kuroda S, Mizutani M, et al. Generalized glycogen storage disease in Japanese quail (*Coturnix coturnix japonica*). *Vet Pathol* 1983;20:312-321.
104. Braga IS, Oda K, Kikuchi T, et al. A new inherited muscular disorder in Japanese quails (*Coturnix coturnix japonica*). *Vet Pathol* 1995;32:351-360.
105. Shane SM, Jaffery MS. Hydropericardium-hepatitis syndrome (Angara disease). In: Calnek BW, ed. *Diseases of poultry*. 10<sup>th</sup> ed. Ames, Iowa: Iowa State University Press, 1997;1019-1022.
106. Cardona CJ, Bickford AA, Galey FD, et al. A syndrome in commercial turkeys in California and Oregon characterized by a rear-limb necrotizing skeletal myopathy. *Avian Dis* 1992;36:1092-1101.
107. Brown MW, Walkinson JH. An automated fluorimetric method for the determination of nanogram quantities of selenium. *Anal Chim Acta* 1977;89:29-35.
108. Craig AM, Blythe LL, Lassen ED, Rowe KE, et al. Variations of serum vitamin E, cholesterol, and total serum lipid concentrations in horses during a 72-hour period. *Am J Vet Res* 1989;50(9):1527-1531.
109. Sheehy PJA, Morrissey PA, Flynn A. Influence of dietary  $\alpha$ -tocopherol on tocopherol concentration in chick tissues. *Brit Poultry Sci* 1991;32:391-397.
110. Gallo-Torres HE. Transport and metabolism. In: Machlin LJ, ed. *Vitamin E: a comprehensive treatise*. New York: Marcel Dekker, Inc., 1980:193-267.
111. Schweigert F, Uehlein-Harrell S, Hegel G, Wiesner H. Vitamin A (retinol and retinyl esters,  $\alpha$ -tocopherol and lipid levels in plasma and captive wild mammals and birds. *J Vet Med A* 1991;38:35-42.

112. Jortner BS, Meldrum JB, Domermuth HD, Potter LM. Encephalomalacia associated with hypovitaminosis E in turkey poults. *Avian Dis* 1985;29:488-498.
113. Jensen JM, Hicks K, Westerman E. Mean serum values of vitamin A and vitamin E in ostrich and emu. In: Jensen JM, Johnson JH, Weiner ST, eds. *Husbandry and medical management of ostriches, emus and rheas*. Wildlife and Exotic Animal TeleConsultants 1992:91.
114. Dierenfeld ES, Traber MG. Vitamin E status of exotic animals compared with livestock and domestics. In: Packer L, Fuchs J, eds. *Vitamin E in health and disease*. New York: Marcel Dekker, Inc., 1992:345-370.
115. Rae M. Degenerative myopathy in ratites. In *Proceedings*. Annu Conf Assoc Avian Veterinarians 1992;328-335.
116. *Mazuri Ostrich and Ratite Diets Product Information & Feeding Guide*. St. Louis, Missouri: PMI, Inc. (Purina Mills), 1993.