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NATURALLY OCCURRING SECONDARY NUTRITIONAL HYPERPARATHYROIDISM IN CATTLE EGRETS (*BUBULCUS IBIS*) FROM CENTRAL TEXAS

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ABSTRACT: Naturally occurring secondary nutritional hyperparathyroidism is described in the nestlings of two colonies of cattle egrets (*Bubulcus ibis*) from Central Texas (Bryan and San Antonio, Texas, USA). Nestlings from a third colony (Waco, Texas, USA) were collected in a subsequent year for comparison. Birds from the first two colonies consistently had severe osteopenia and associated curving deformities and folding fractures of their long bones. These birds also had reduced bone ash, increased osteoclasia, a marked decrease in osteoblast activity, variable lengthening and shortening of the hypertrophic zone of the epiphyseal cartilage, decreased and disorganized formation of new bone, and a marked hypertrophy and hyperplasia of the parathyroid glands as compared to birds collected from the third colony. Fibrous osteodystrophy was found in all of the birds from San Antonio and Bryan. Evidence of moderate to severe calcium deficiency was also identified in 33% of the cattle egrets collected from Waco. Gut contents of affected chicks contained predominately grasshoppers and crickets; vertebrate prey items were absent from the Bryan birds. Grasshoppers and crickets collected from fields frequented by the adult egrets in 1994 had 0.12–0.28% calcium and 0.76–0.81% phosphorus. Pooled grasshoppers and crickets collected during a subsequent wet early spring averaged 0.24% calcium and 0.65% phosphorus. Although the phosphorus content of the insect prey was adequate for growth, calcium was approximately one-third the minimum calcium requirement needed for growth for other species of birds. It was postulated that cattle egrets breeding in Central Texas have expanded their range into habitat that contains less vertebrate prey, and as a result, many nestling egrets are being fed diets that contain suboptimal calcium. Therefore, in years where vertebrate prey is scarce and forage for insect prey is reduced in calcium, nestling egrets are at risk for developing secondary nutritional hyperparathyroidism.

Key words: *Bubulcus ibis,* cattle egrets, secondary nutritional hyperparathyroidism.

INTRODUCTION

Abnormal mineralization of growing bone is a manifestation of diets containing excessive calcium and inadequate phosphorus (Long et al., 1984a), inadequate calcium (Long et al., 1984b), inadequate vitamin D_3 (Long et al., 1984c), or inadequate calcium and excess phosphorus (MacWhirter, 1994). Secondary nutritional hyperparathyroidism, as a consequence of insufficient dietary calcium or excessive phosphorus, is a commonly encountered problem in captive-raised exotic birds, particularly captive nestling birds of prey fed unsupplemented all-meat diets (Wallach and Flieg, 1969; Cooper, 1975; Long et al., 1983), and nestling cage birds fed homemade formulas high in cereal grains (MacWhirter, 1994).

Bone disorders resulting from mineral imbalances are rare in wild birds. To the authors' knowledge, only two such cases have been reported. The first report describes nestling vulture chicks (*Gyps* sp.) from South Africa with pathological fractures. These birds were only fed meat instead of meat containing crushed bone, as bone crushing carnivores were no longer present in the birds' range (Evans and Piper, 1981; Richardson et al., 1986). The second case of secondary nutritional hyper-

parathyroidism involved fledgling American crows (*Corvus brachyrhynchos brachyrhynchos*) in Long Island, New York, USA (Tangredi and Krook, 1999). The cause of this disease was not determined but was speculated to be the result of crows feeding on human-generated garbage that may have contained insufficient calcium, excess phosphorus, or both. Vitamin D_3 deficiency may have also played a role in the disease in these birds.

This report describes naturally occurring secondary nutritional hyperparathyroidism in nestling and fledgling cattle egrets (*Bubulcus ibis*) in two breeding colonies in central Texas during the 1994 breeding season. Histological findings of the bone and parathyroids and percent bone ash were compared to cattle egrets collected from a third colony in 1997, in which signs of nutritional hyperparathyroidism were not observed.

MATERIALS AND METHODS

Approximately 200 nestling and fledgling cattle egrets (age 1–6 weeks) that were presented to the Veterinary Teaching Hospital (College of Veterinary Medicine, Texas A&M University, College Station, Texas, USA) by members of the public from 25 June to 5 August 1994 were included in this study. All of these birds were found on the ground within 100 m of a cattle egret breeding colony (approximately 10,000 birds) on the west edge of the city of Bryan, Texas, USA (30°40′N, 96°22′W). Four additional birds were collected at a cattle egret colony in San Antonio, Texas, USA (29°25′N, 98°29′W) on 4 August 1994 by one of the authors (Contreras) in an investigation into a report of dead and dying birds at this location. Thirty outwardly normal, age-matched, cattle egret nestlings and fledglings were collected on 1 July 1997 at a third colony located in Waco, Texas, USA (31°36′N, 97°13′W).

All birds were examined thoroughly. Three birds from the Bryan colony with characteristic signs (lameness and drooped wing) were radiographed. Five birds, also with characteristic signs, from the Bryan colony, and 30 birds from the Waco colony were necropsied at Texas A&M University. The four birds collected from the San Antonio colony were submitted for necropsy to the Texas Veterinary Medical Diagnostic Laboratory (College Station, Texas, USA). Tissues were fixed in 10% buffered-formalin, dehydrated, embedded in paraffin, and sectioned at 6 μm. Deparaffinized sections were stained with hematoxylin and eosin. Bone was demineralized in 10% disodium ethylenediaminetetraacetic acid for 14 days and similarly processed. Complete sets of tissues, including longitudinal sections through the proximal tibiotarsus, fractured bones, and parathyroid were examined microscopically from all of the egrets collected in Bryan. Sections of parathyroid gland from the five egrets from Bryan, but only 27 of the egrets from Waco, included sufficient parathyroid gland to interpret. Bone was only examined microscopically from two of the four birds from San Antonio. The parathyroid glands of these birds were not examined.

The process of bone formation was evaluated on each cross section of tibiotarsus, and each section was given a score of from 1 to 5. Sections with a severe interruption of bone formation were given a score of 1, and sections with normal bone formation (some Waco birds) were given a score of 5. Bone scores were determined by length of the hypertrophic zone, length and width of newly formed osteoid seams, presence or absence of an organized transition to mineralized bone from cartilage, width and number of trabeculae within the medulla of the bone, osteoblast activity, and presence of fibrosis.

The average number of osteoclasts per field (20× objective) in the zone of provisional mineralization was determined as follows. A 1 cm^2 grid was placed in an ocular. The grid was lined up so that its outside edge was midway between the cortical bone and the first trabecula within the medulla. Osteoclasts were defined as those cells with multiple nuclei and abundant eosinophilic cytoplasm. Cells that may have been osteoclasts but only had a single nucleus were not counted. All the osteoclasts completely within the grid were counted. Osteoclasts touching the right and upper grid line were also counted. Osteoclasts touching the left or lower grid lines were not counted. Adjacent sections of the bone were examined until the opposite cortex was reached. Three or four grid fields were examined in each bone section. The average number of osteoclasts per grid field was then calculated. The degree of parathyroid hypertrophy and hyperplasia was graded on a scale of 1–5. A score of 1 indicated that there was no hypertrophy or hyperplasia of the parathyroid gland, and a score of 5 indicated that the hypertrophy and hyperplasia of the gland were severe.

A complete set of tissues, including kidney, duodenum, colon, and four or more sections of the jejunum were also examined.

Ash content of a tibiotarsus was determined

for two of the cattle egrets collected in San Antonio and 28 of the egrets collected from Waco at the Texas Veterinary Medical Diagnostic Laboratory (College Station, Texas, USA). Briefly, bone was stripped of all associated soft tissue, and the epiphysis was cut from either end of the bone and discarded. The diaphysis was longitudinally sectioned, and the marrow was removed by scraping the internal surface of the cortex. Bones were then defatted in acetone, air dried, and weighed. Bones were ashed overnight at 600 C, and the ash was weighed. The percentage ash content of the bone was calculated by dividing the weight of the ash by the weight of the defatted bone and multiplying by 100. Plasma calcium concentrations were evaluated (Vitro 250, Ortho Clinical Diagnostics, Rochester, New York, USA) on four birds from the Bryan colony and 29 birds from the Waco colony.

The proventricular and ventricular contents of 20 additional birds from the Bryan colony and all 30 birds from the Waco colony were examined. During dissection of 20 birds from the Bryan colony, four had enlarged green livers. Aseptic samples of the diseased livers were plated on blood and MacConkey's agar and incubated overnight at 38 C. Routine biochemical assays were used to identify bacterial isolates. Slide agglutination using group specific antibody (Diffco, Detroit, Michigan, USA) was used to determine whether isolates were group B salmonella. Abnormal livers were also evaluated histologically. Intestinal contents of all of the birds from the San Antonio colony and from the liver and spleen of a single San Antonio bird were also cultured.

Crickets and grasshoppers were collected from fields in which cattle egrets were seen to be feeding during the month of July 1994, and again in these same fields in June 2001. Field and tree crickets (Gryllidae), and long-horned (Tettigonidae) and short-horned (Acrididae) grasshoppers, collected in 1994, were analyzed for their calcium and phosphorus content using the standard Hatch protocols: sample digestion method number 00.30, calcium determination method number 00.60, and phosphorus determination method number 00.50 (Hatch Company, Loveland, Colorado, USA) at the Texas Veterinary Medical Diagnostic Laboratory (Amarillo, Texas, USA). Voucher specimens of these crickets and grasshoppers were deposited with the Department of Entomology (Texas A&M University, College Station, Texas, USA). Equal volumes of crickets and grasshoppers collected in June 2001 were pooled and analyzed by the same method.

Daily, monthly, and average rainfall totals for the months of May, June, and July for San Antonio and Bryan in 1994, May and June for Waco in 1997, and May and June for Bryan in 2001 were obtained from the office of the Texas State Climatologist (Department of Meteorology, Texas A&M University, College Station, Texas, USA).

RESULTS

All three egret colonies were located in or immediately adjacent to a residential area. The Waco colony was within 200 m of the Brazos River. Both the San Antonio and Bryan colonies had been nesting in the same vicinity since the colonies were established in 1990. The Waco colony had only been in its current location for the 1996 and 1997 breeding seasons.

Fledgling and nestling cattle egrets from the Bryan colony, beginning the last week of June 1994 and continuing into the second week of August 1994, were found on the ground unable to fly. Many of these birds were limping or completely unable to walk, and most drooped one or both wings. The precise number of birds affected was not known; however, over 3,000 dead birds were picked up by the City Sanitation Department in Bryan (Brown, pers. comm.). Observations at the San Antonio colony were made only once, on 4 August 1994. At this time, 100 or more dead juvenile birds were found on the ground. Some juvenile birds, still in the trees, were observed to have difficultly perching. Live birds on the ground were listless and unable to walk more than a few steps at a time. A minimal number of dead fledgling and nestling egrets (<10% of the number seen at the Bryan colony) were present on the ground and in the trees at the Waco colony. All birds collected from the Waco colony were from nests or adjacent branches.

Approximately 200 egrets from the 1994 Bryan colony were presented to the Veterinary Teaching Hospital. Most were fully feathered fledglings, but some were nestlings. The nutritional status of these birds ranged from complete emaciation, with wasting of the pectoral muscles and absence of body fat, to well-muscled birds

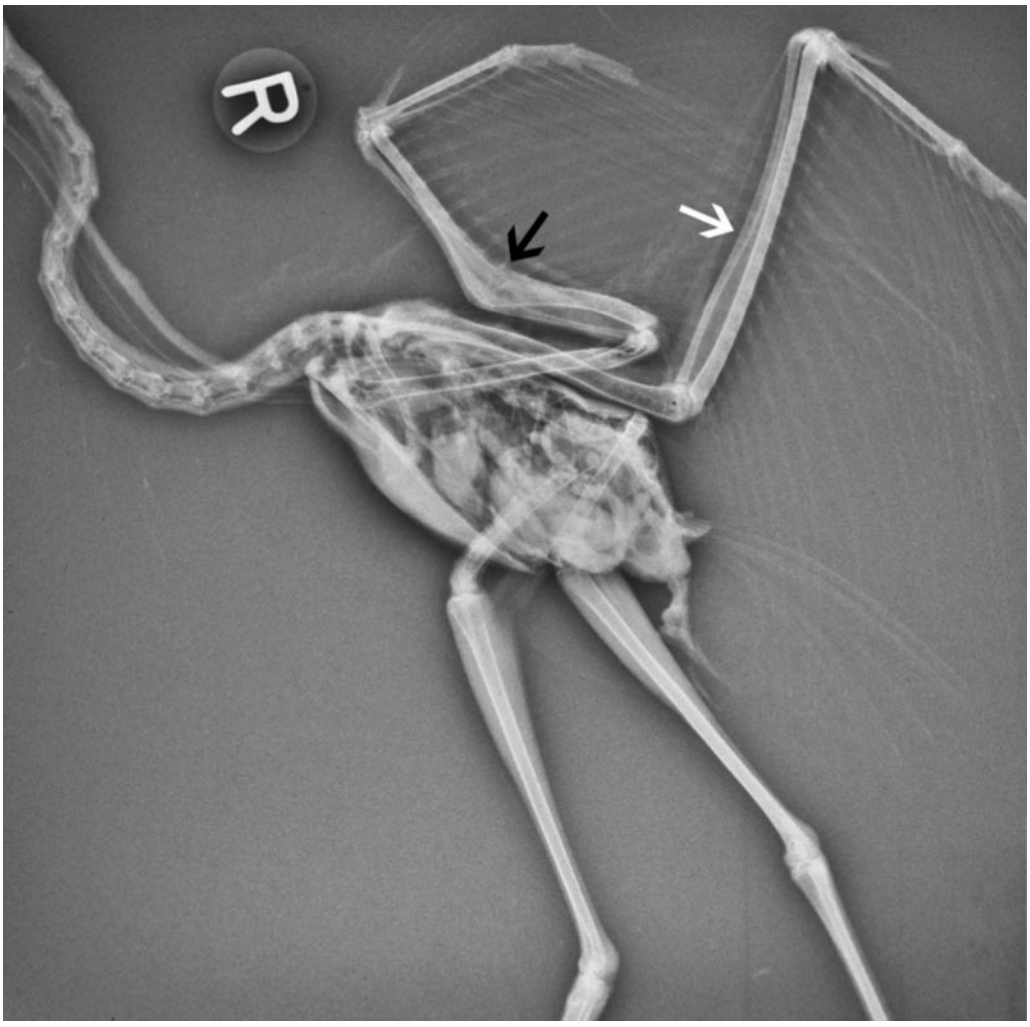

FIGURE 1. Lateral radiograph of a fledgling cattle egret with secondary nutritional hyperpararthyroidism. The bird was unable to fly. There are folding fractures of the right ulna and radius (black arrow) and a curving deformity of the left radius (white arrow).

with substantial body fat. All birds had signs of bone disease. Most had fractures of one or both wings and legs. All bones were soft, and even gentle restraint often resulted in additional fractures. Radiographs revealed a generalized reduction in mineral density of all bones. Curving deformities of the long bones, particularly the radius, were common. Long-bone fractures were present in all four of the radiographed birds (Fig. 1). Delayed and asymmetrical pneumatization of the humeri was also a consistent finding.

Seven carcasses were examined from the Bryan and San Antonio colonies in 1994. All birds had osteopenic disease characterized by pliable to fragile skeletons. There were multiple pathological fractures. Some fractures included obvious swellings composed predominantly of grossly discernible hyaline cartilage. Fractures were sometimes combined with curving deformities of long bones. Many ribs were similarly deformed by bending as well as by angulated, malaligned pathological fractures. The process of fracture repair had begun at most of the grossly observed fracture sites but was interrupted

at the stage of primary (cartilage) callus formation.

Midsagittal sections of a tibiotarsus from five of the birds from Bryan and two of the birds from San Antonio were examined. All birds had marked abnormalities of the epiphysis. Five of the birds had either moderate lengthening or moderate shortening of the zones of hypertrophic and mineralizing cartilage. Axial osteoid developed at varying levels within the zones of hypertrophic and mineralizing cartilage. In addition, these seams were thin and discontinuous. Islands of mineralized cartilage persisted into the provisional zone of calcification, resulting in the formation of transverse osteoid seams (Figs. 2, 3). In one bird, the zone of hypertrophic cartilage was severely shortened, and there was no provisional zone of mineralization. The medullary canal was filled with marrow but did not contain trabeculae (Fig. 2). The last bird had an elongate epiphysis. Mineralization and osteoid deposition at the end of the elongate zone of hypertrophic cartilage was reduced and poorly organized. Another more orderly zone of provisional mineralization was also present midway between the first zone of provisional mineralization and the distal edge of the zone of hypertrophy. Scalloped edges of trabeculae as the result of increased osteoclasia were common. Active bone deposition, as judged by osteoblast activity, was minimal to none. Fibrous osteodystrophy was seen in all seven birds (Fig. 4).

Gross evidence of bone disease was not seen in birds collected from Waco. A range of histological patterns was found in the tibiotarsal sections from the Waco birds. At one extreme, where bone formation was judged normal, the hypertrophic zone of cartilage was long, and vessels penetrated to the level of the zone of proliferating cartilage. Osteoid seams began deep in the hypertrophic cartilage zone, and they were straight, parallel with each other, and relatively wide. They extended, with few interruptions, into the diaphysis. The zone of provisional calcification was relatively short, and there was an orderly transition between mineralized cartilage and trabecular bone (Figs. 2, 3). Surrounding the newly forming trabeculae were densely packed plump osteoblasts (Fig. 4). At the other extreme, the hypertrophic zone was variable in length. In some birds, it was up to 50% longer than the apparently normal birds, and in others it was somewhat shorter (Fig. 2). Zones of hypertrophic and degenerate cartilage had irregular margins, and the vascular spaces tended to be wider. Osteoid seams either did not penetrate into the hypertrophic zone, or did so erratically. Osteoid seams were thin and frequently interrupted. Curved and transverse osteoid seams also were seen. Transition from mineralized cartilage to trabeculae occurred at various distances into the bone, and cartilage islands persisted deep into the diaphysis. Trabeculae were reduced in number and were thin. Osteoblasts were fewer in number, had less cytoplasm, and were more likely to be flattened against the trabeculae (Fig. 4). Fibrous osteodystrophy was observed in three (10%) of the birds collected in Waco.

Parathryoid glands of all the egrets from Bryan (n=5) were enlarged. Parathyroids from the Waco birds ranged from not visible (12 birds) to small (16 birds) to enlarged (three birds). Parathyroid gland chief cells of all egrets from the Bryan colony showed a prominent degree of hypertrophy and clear vacuolation of the cytoplasm, resulting in a marked decrease in the nuclear to cytoplasmic ratio. Cells with vacuolated cytoplasm resembling water clear cells described in mammals were moderately common to abundant. Seven Waco birds had normal parathyroids, indicating that their diet contained adequate calcium. Mild parathyroid hypertrophy, indicating a diet with marginal calcium, was present in 13 egrets from Waco. Seven birds had significant hypertrophy of their parathyroids, indicating that they were being fed a calcium-deficient diet. Vacuolat-

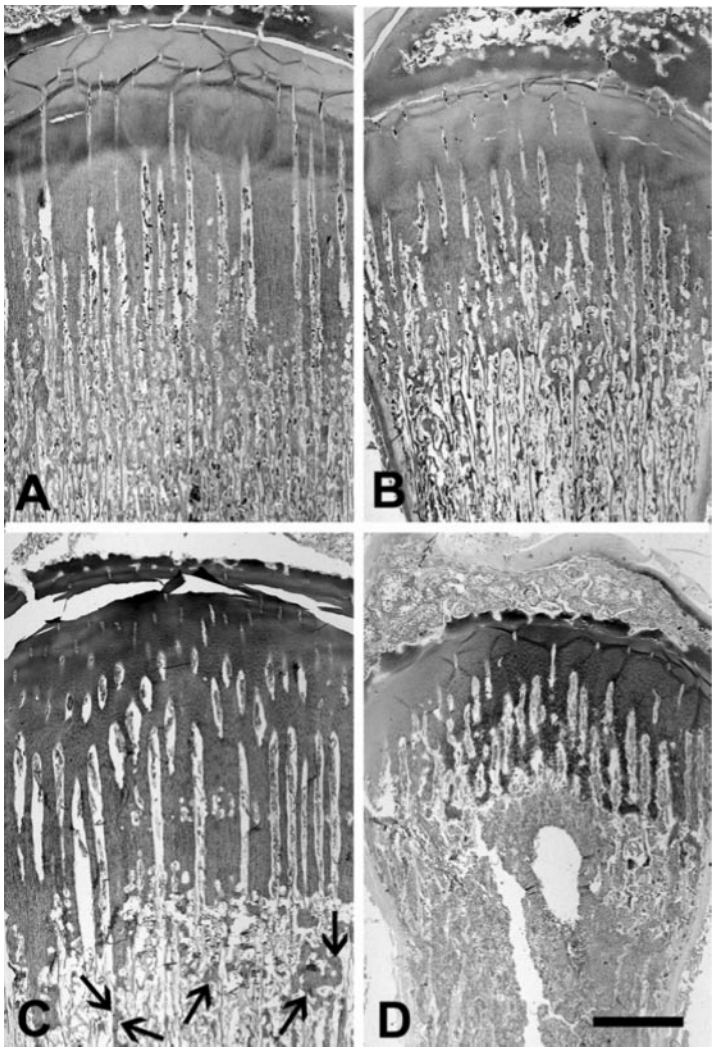

FIGURE 2. Longitudinal section through the proximal tibiotarsus of four fledgling cattle egrets stained with hematoxylin and eosin. Normal growth plate (A), egret from the Waco colony. Note the cartilage columns are straight with parallel sides and that there is an organized transition from cartilage to mineralizing bone. Egret from the Waco colony with secondary nutritional hyperparathyroidism (B). The hypertrophic zone of cartilage is slightly longer than the normal bird. The cartilage columns fuse and have irregular edges, the zone of primary calcification is irregular, and newly formed osteoid seams are thin and discontinuous. Vascular channels are widened. Egret from the Bryan colony with secondary nutrition hyperparathyroidism (C). The zone of primary calcification is similarly irregular, and osteoid seams are thin. In addition, there is persistence of islands of cartilage into the diaphysis (arrows). A second egret with secondary nutritional hyperparathyroidism from the Bryan colony (D). The proliferating and hypertrophic zones of cartilage are markedly shortened, and formation of new trabeculae has stopped. Trabeculae are not found in the medullary cavity. Bar = 3 mm.

ed cells were present in birds with parathyroid scores of 3 and 4. The numbers of vacuolated cells were highest in birds with parathyroid scores of 4. The number of vacuolated cells was always less then the numbers found in the parathyroid glands of egrets from Bryan (Fig. 5).

Bones (n=7) from the Bryan and San Antonio birds were all scored as 1. The bone scores for Waco birds ranged from 1

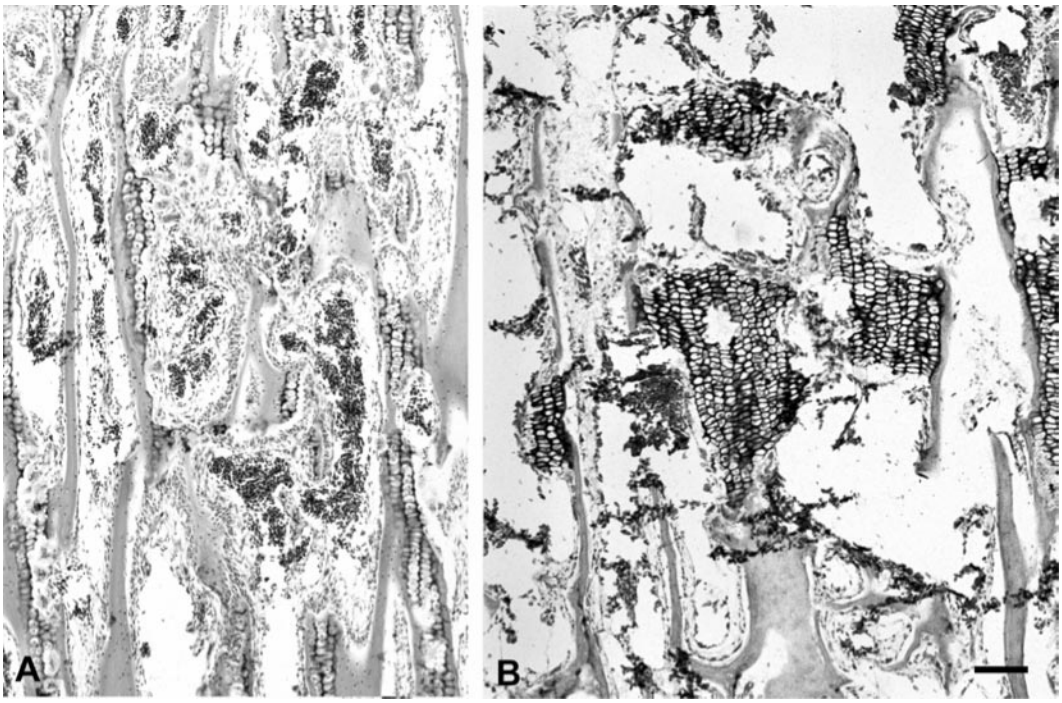

FIGURE 3. Higher magnification of zone of preliminary calcification from an egret with normal bone formation (A) and an egret with secondary nutritional hyperparathyroidism (B). Note the retained islands of degenerate cartilage cells in the bird with secondary nutritional hyperparathyroidism. Bar = 120 μm.

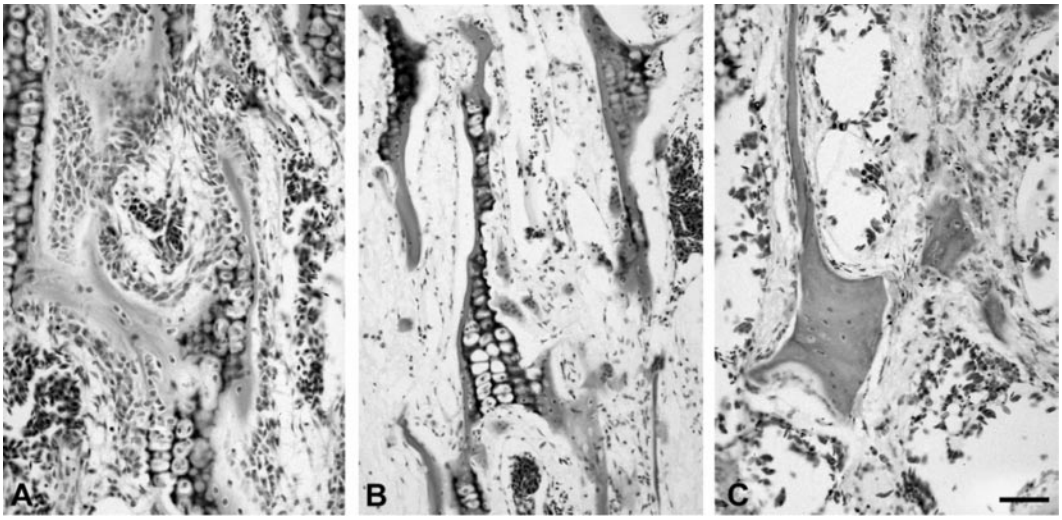

FIGURE 4. Trabeculae from an egret with normally forming bone (A) and two egrets [one from the Waco colony (B) and one from the Bryan colony (C)] with secondary nutritional hyperparathyroidism. Plump osteoblasts surround enlarging trabeculae in the normally forming bone. In the birds with hyperparathyroidism, there is peritrabecular fibrosis, and the osteoblasts lay flattened against the bone. Trabeculae are typically thin, and in section B there are several osteoclasts and the trabecular margins are scalloped, indicating bone resorption. Bar = 30 μm.

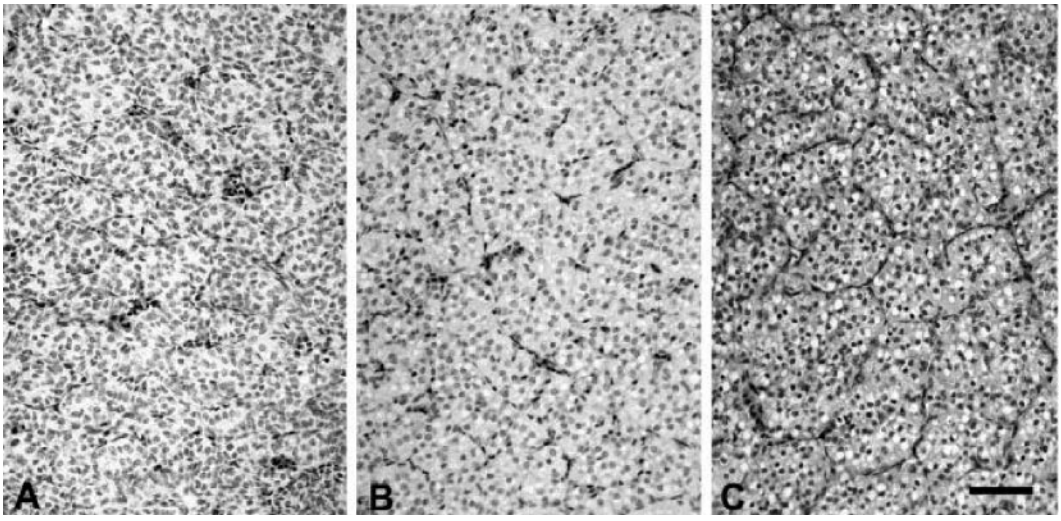

FIGURE 5. Sections of a parathyroid gland from an egret with normally developing bone (A) and the two egrets with fibrous osteodystrophy (B [an egret from Waco] and C [an egret from Bryan]). In panel A, the nuclear-to-cytoplasmic ratio is approximately 1:1. In panels B and C, the parathyroid cells have hypertrophied, causing separation of the nuclei and a decrease in the nuclear to cytoplasmic ratio. Vacuolated cells are absent in the parathyroid from the bird with normal bone, sporadically present in parathyroid from the egret from Waco, and numerous in the parathyroid gland from the egret from College Station. Bar = 50 μm.

to 5, with 50% of the birds having a bone score of 5 (Fig. 6). Ten birds (33%) had significant alterations in bone growth (bone scores of 3 or less). The median number of osteoclasts per grid field for Bryan egrets ranged from six to 82, with a median value of 53 (n=7). The value of six came from a bird that had very few trabeculae. The median number of osteoclasts per grid field for Waco egrets was 28, with a range of two to 89 (n=28) (Fig. 7). All the birds from Bryan had a parathyroid score of 5. Seven birds from the Waco colony (26.9%) had parathyroid scores of 3 or 4, indicating a persistent suboptimal insufficiency in dietary calcium (Fig. 8).

Bone ash values from two egrets collected from San Antonio were 42.7 and 48% (Fig. 9). Bone ash (n=28) from egrets collected in Waco ranged from 35 to 78%, with a mean value of 58%. Birds with bone scores of 1 had bone ash values of 51, 53, and 57%. There was an incomplete correlation between bone score and bone ash. The lowest bone ash (38%) was from a bird that had a bone score of 5. The highest bone ash (78%) was in a bird with a bone score of only 2. Plasma calcium concentrations in five egrets from Bryan were 7.0, 7.9, 8.2, 8.4, and 9.9 mg/dl. Plasma calcium concentrations in egrets from Waco ranged from 6.0 to 11.6 mg/dl (n=29) (Fig. 10). Birds with bone scores of 1 had calcium concentrations of 7.7, 7.6, and 6.6 mg/dl.

Comparing values obtained for egrets from Waco and egrets from Bryan and San Antonio, the bone scores of birds from Bryan and San Antonio were always less. Fibrous osteodystrophy was seen in all egrets from Bryan and San Antonio, but only in three egrets from Waco. Five of five egrets (100%) from Bryan had gross enlargement of the parathyroids as compared to only three of 30 (10%) egrets from Waco. Likewise, birds from Bryan always had a greater degree of microscopic parathyroid hyperplasia and hypertrophy as compared to egrets from Waco. With the exception of the one bird that did not have trabeculae, the number of osteoclasts per grid field for birds from Bryan and San Antonio fell into the top 30th percentile of

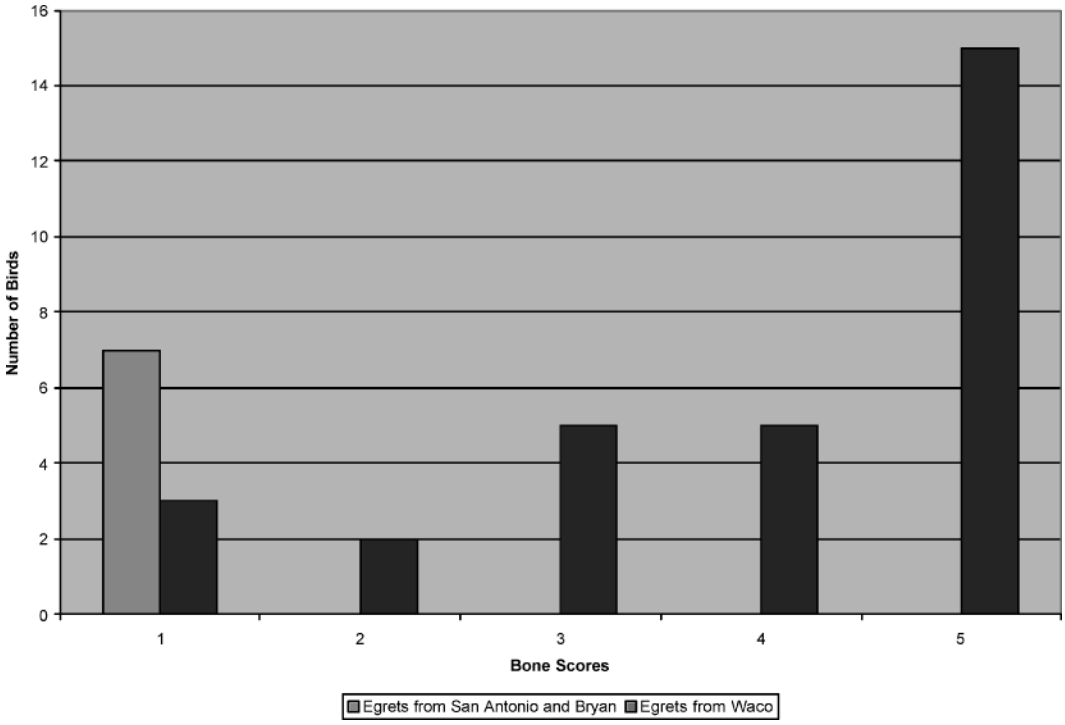

FIGURE 6. Distribution of bone scores from egret chicks from the Bryan, San Antonio, and Waco, Texas, USA.

all egrets examined, whereas the bone ash values of the two San Antonio birds were in the lowest 10th percentile of all birds examined.

Lesions were not found in the gastrointestinal and renal tissues examined from the Bryan and San Antonio egrets. Moderate to severe subacute hepatitis with intralesional bacteria was found in the four egrets from the Bryan colony with enlarged and discolored livers, and in one egret from the San Antonio colony. A group B *Salmonella* was grown in pure culture from liver of all five of these birds. A *Salmonella* sp. was also isolated from the intestinal contents of two of the remaining three San Antonio birds.

Proventricular and ventricular contents of 20 egret chicks collected from the Bryan colony in 1994 consisted exclusively of insects. Although not quantitated precisely, 80% or more of the volume of the ingesta was composed of crickets and grasshoppers in all but one bird. In the remaining bird, rat-tailed maggots (*Eristalis* sp.) were the only prey species found. Ingesta of the Waco cattle egrets were also found to be more than 80% crickets and grasshoppers. A partially digested frog was found in the stomach of one bird. In addition, a partially digested mouse was found in regurgitus from another egret that was not collected.

Calcium concentration of field crickets and two genera of grasshoppers collected during July 1994 were very low, ranging from 0.12% to 0.14%. The calcium concentration in the tree crickets was twice as high but was still low (0.28%). The concentration of calcium in the pooled crickets and grasshoppers from May 2001 was 0.25%. Phosphorous concentrations from all groups were similar (range 0.65–0.81%). The calcium-to-phosphorus ratio was lowest in the grasshoppers collected in July 1994 and highest in the pooled crickets and grasshoppers collected in May

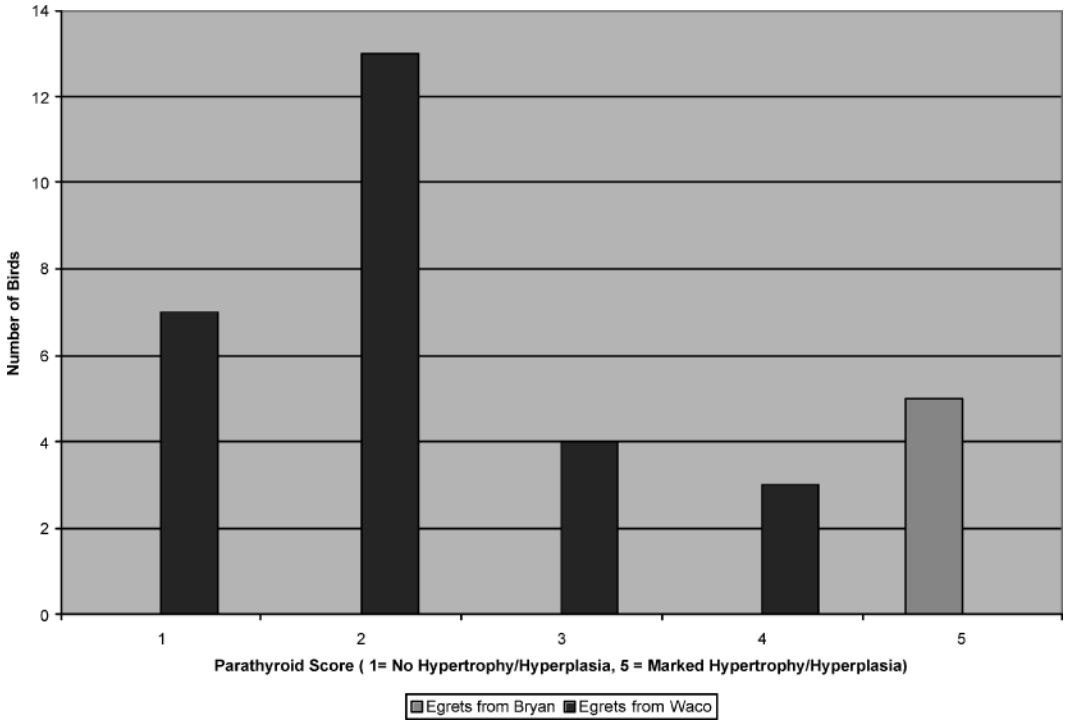

FIGURE 7. Distribution of the average number of osteoclasts per grid field of the proximal tibiotarsus. Egret checks from Bryan, San Antonio, and Waco, Texas, USA.

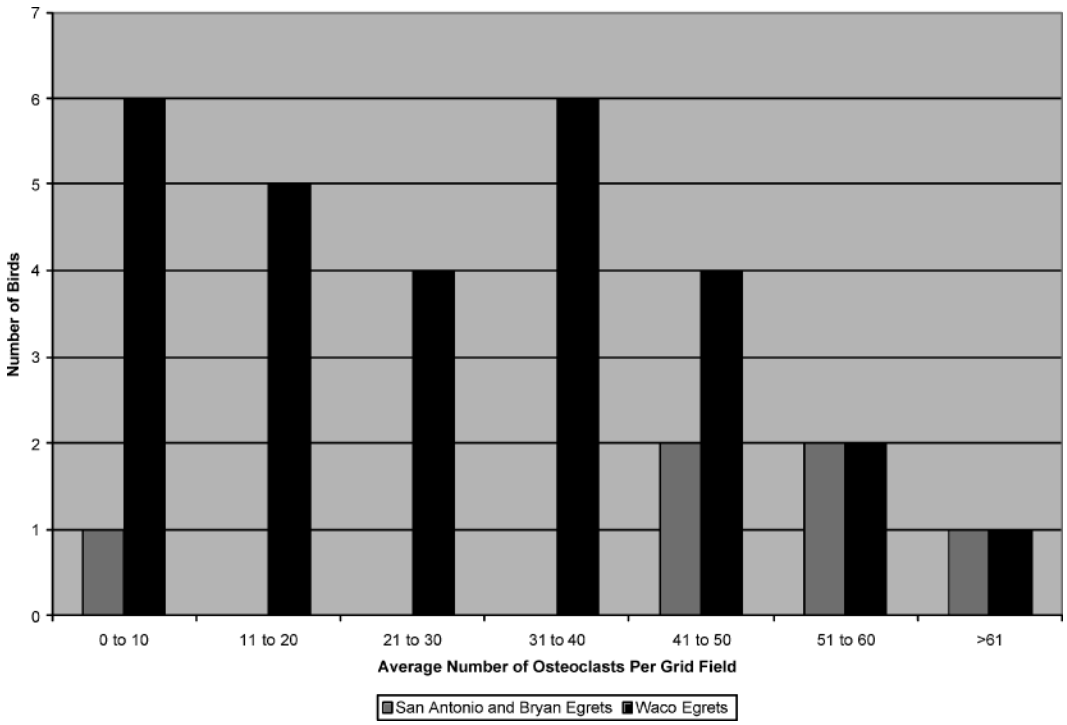

FIGURE 8. Distribution of parathyroid scores of egrets chicks from Bryan and Waco, Texas, USA.

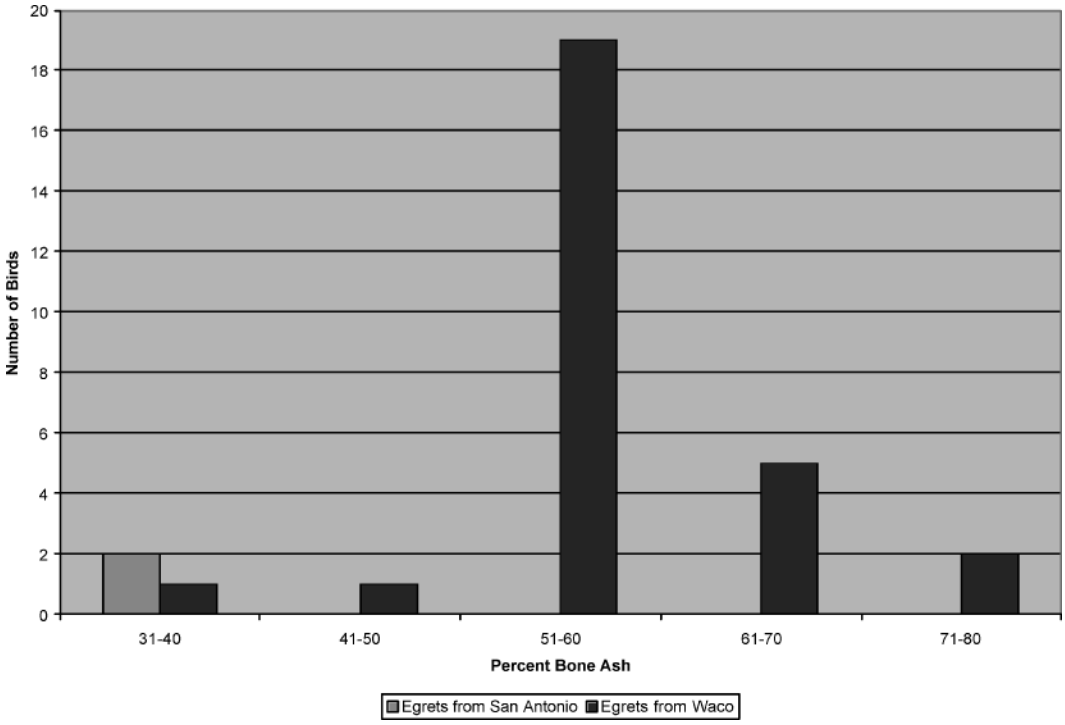

FIGURE 9. Distribution of percent tibiotarsal bone ash of egret chicks from San Antonio and Waco, Texas, USA.

2001 and tree crickets collected in July 1994 (Table 1).

The actual and expected rainfalls during May, June, and July for Bryan and San Antonio are listed (Table 2). In Bryan, 6.86 cm of the total 9.30 cm of rain for the month of June fell during the first 11 days of the month. This meant that the second half of June and all of July and August were below the expected rainfall. The rainfall for Bryan during the months of May and June of 2001 is also listed. Rainfall in June was above average, and the pastures from which grasshoppers and crickets were collected were rapidly growing.

DISCUSSION

The bone and parathyroid lesions found in the egret chicks from Bryan and San Antonio were characteristic of secondary hyperparathyroidism. Parathyroid glands had marked hypertrophy, hyperplasia, and vacuolization of the chief cells, the later change being consistent with prolonged parathyroid hormone secretion (Harach, 2004). The birds had fragile bones that contained multiple deformities and pathological fractures. Histologically, fibrous osteodystrophy, a characteristic lesion of hyperparathyroidism, was prominent. Changes in the growth plates were variable but resembled those seen in experimentally induced hyperparathyroidism in chickens fed low-calcium diets (Long et al., 1984b). The low bone ash, increased osteoclasia, and relatively low plasma calcium, seen in three of the four egret chicks from Bryan, were also consistent with hyperparathyroidism (Hurzitz and Griminger, 1961).

Secondary hyperparathyroidism can be the result of diets deficient in calcium, diets with sufficient calcium and excess phosphorus, or diets deficient in vitamin D_3 (Tangredi and Krook, 1999). High-fat diets can interfere with vitamin D_3 absorption and cause vitamin D_3 deficiency.

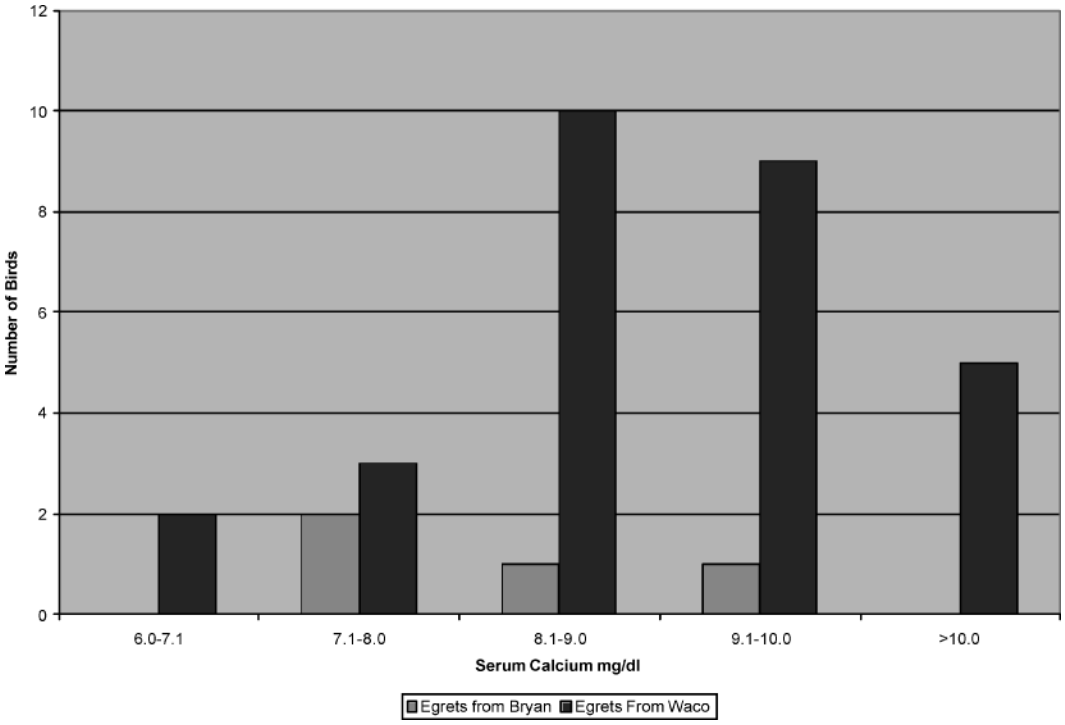

FIGURE 10. Distribution of plasma calcium concentrations of egret chicks from Bryan and Waco, Texas, USA.

Enteritis can interfere with calcium absorption (Perry et al., 1991), and severe kidney disease can result in the increased loss of calcium; both may result in secondary hyperparathyroidism (Tangredi and Krook, 1999). Experimentally, poisoning with ochratoxin A has also been shown to cause bone lesions that resemble secondary nutritional hyperparathyroidism (Duff et al., 1987).

Nutritionally related bone disorders in wild birds are rare, and in the two cases reported they were thought to be the result of manmade alterations in the birds' environment. Bone disease in Gyps vultures appears to be the result of the decline in bone-crushing carnivores. As a result of this decline, vultures only have access to meat instead of meat containing crushed bone (Richardson et al., 1986). Muscle contains excessive phosphorus and insufficient calcium and an all-meat diet

TABLE 1. Calcium and phosphorus concentrations (on a dry matter basis) and the calcium and phosphorus ratios of crickets (Gryllidae) and grasshoppers (Tettigonidae and Acrididae) forming the predominate food items of the egrets with secondary nutritional hyperparathyroidism.

Prey species	Calcium (%)	Phosphorus (%)	Calcium-to-phosphorus ratio
Field crickets (Gryllidae), July 1994	0.14	0.76	0.18
Tree crickets (Gryllidae), July 1994	0.28	0.81	0.35
Long-horned grasshoppers (Tettigonidae), July 1994	0.12	0.74	0.16
Short-horned grasshoppers (Acrididae), July 1994	0.12	0.74	0.16
Pooled crickets and grasshoppers, May 2001	0.25	0.65	0.35

TABLE 2. Average and actual rainfall (cm) May through August 1994, Bryan and San Antonio, Texas; May and June 1997, Waco, Texas; and May and June 2001, Bryan, Texas.

	May	June	July
San Antonio			
Average	10.72	9.70	5.50
1994	17.80	4.22	1.27
Bryan			
Average	12.19	9.35	5.82
1994	14.14	9.30[a]	0.28
2001	12.22	16.02	Not Applicable
Waco			
Average	11.63	8.33	Not Applicable
1997	8.92	6.88	Not Applicable

[a] Of the total of 9.30 cm that fell in Bryan during June, 6.86 cm fell in the first 11 days.

would have resulted in the failure of bone mineralization in vulture chicks. Bone growth disorders reported in American crow nestlings on Long Island, New York, USA, appeared to result from a diet deficient in calcium and possibly in excess of phosphorus (Tangredi and Krook, 1999). Crows, being opportunistic feeders, were suspected of feeding their nestlings scraps of food from human refuse that did not provide a proper mineral balance for their chicks. It was also postulated that this diet might have been deficient in vitamin D_3 or that vitamin D_3 metabolism may have been interfered with by something in the diet.

Analysis of the prey fed to the cattle egret nestlings from Bryan strongly indicated that bone disease observed in these birds was a natural phenomenon resulting from a diet deficient in calcium. Insects, predominately grasshoppers and crickets, were the only prey item found in their stomach contents. Calcium and phosphorus analysis of these insects showed them to contain an adequate level of phosphorus (0.6%) but insufficient calcium (0.1–0.28%) as compared to that necessary for normal bone growth in ducks (0.65%), chickens (0.7%) (Shane and Young, 1969), and turkeys (1.2%) (National Research Council, 1994). The budgerigar (*Melopsittacus undulates*), and possibly other seed-eating birds, may require as little as 0.3% dietary calcium for normal bone growth (Roset et al., 2000), but this is still significantly more calcium then what the egret chicks were being fed. Vitamin D_3 deficiency would not occur in birds fed plant-eating insects and exposed to natural light. In addition, neither enteric nor renal lesions, other potential causes of secondary hyperparathyroidism, were found in these birds. Hepatitis was found in several birds, but not in all, and the hepatic lesions were acute and the bone lesions chronic. Ochratoxin A–induced lesions are characterized by widened and less numerous metaphyseal vessels, a lesion not seen in the egrets in this study (Duff et al., 1987).

In the short collection trip to the Waco colony, egrets with signs of secondary nutritional hyperparathyroidism were not observed. Therefore, it was assumed they would have normal bone growth and mineralization and would serve as controls for the egrets collected from the Bryan and San Antonio colonies. Although most birds did have normally growing bones, 30% showed varying degrees of secondary nutritional hyperparathyroidism, and 10% of these birds had severe lesions, including fibrous osteodystrophy. These findings indicate that the circumstances that led to the nutritional disease seen in the Bryan and San Antonio egrets may have also been present, to a lesser extent in Waco.

It is impossible to know all the factors that contributed to the nutritional disease observed in the cattle egrets, but we speculate that it may be the result of expansion of the cattle egret into suboptimal habitat. Cattle egrets are newcomers to North America and only arrived in Texas in the late 1940s. Initially, cattle egrets colonized the Texas Coast and river systems, but they have continued to expand their range so that now breeding colonies are present throughout much of Texas, including areas with few wetlands (Telfair, 1983). As these birds moved into drier climates, the avail-

ability of calcium-rich prey may have diminished. Telfair (1983), in an exhaustive review of the cattle egret in Texas, found approximately 30% of the volume of nestling cattle egret diets to be vertebrate prey, and most of the vertebrates eaten were amphibians. The remaining 70% of their diet was insects, predominately grasshoppers and crickets. Amphibians would be expected to be in much greater abundance in coastal and river bottom habitats, as compared to the drier ranch and farm land of Central Texas. Vertebrate prey would be expected to be an important source of calcium for the growing egret. As vertebrate prey was not found in any of 20 egrets from Bryan examined in this study, it was felt that their absence was the major contributing factor toward the development of secondary hyperparathyroidism.

Why adult cattle egrets during this year, and in these locations, were not feeding on vertebrates is not known. However, climatic and geographical factors may have played a role making amphibian prey scarce. During 1994 in both San Antonio and Bryan, excess rain fell in May, but little rain fell from the second week in June and to the end of July. During May, amphibian activity may have been at its peak; in contrast, during the dry months, amphibian activity would have been expected to decrease, making them less accessible to foraging cattle egrets. As chicks first leave the nest approximately 21 days after hatching (Telfair, 1983), all of the egrets in this study hatched between the first week of June and the second week of July, and therefore would have been raised during the dry period.

The location of these egret colonies may have also been a contributing factor to this problem. San Antonio and Bryan have relatively little surrounding wetland, and the abundance of amphibian prey would be expected to be low. The Waco colony, however, was located in the flood plain of the Brazos River and was immediately adjacent to its wetlands. Therefore, even though the birds collected from Waco were also collected following a dry month, their parents may have had better access to amphibian prey. This hypothesis was consistent with the presence of a frog in the ingesta of one egret from Waco.

We cannot rule out the possibility that the absence of rain may have affected the nutrient content of the cattle egret's insect prey and contributed to the secondary hyperparathyroidism. Insects feeding on succulent growing plants might have a higher nutrient value than those feeding on dry, stunted, water-starved plants. Calcium concentrations of pooled grasshoppers and crickets collected during June 2001, when there was sufficient rain, had twice the calcium concentration as the grasshoppers and field crickets collected during the dry summer of 1994. The concentration of calcium in them was, however, still considerably less than that thought to be minimally necessary for nestling growth based on what is known in other species of birds.

Not all of the data collected in this study was as expected. In particular, bone ash was found to be low in one bird from Waco with a high bone score and high in another bird from Waco with a low bone score. These discrepancies may represent birds that were intermittently calcium deprived, so that histology of the growing bone did not represent the state of the remainder of the bone. Alternately, the way the bone ash was determined may have resulted in falsely low values for some birds. One step of this process included scraping the marrow from the medulla of the bone. It is likely that this step also resulted in the dislodging of newly formed trabeculae that would have contained calcium and phosphorous.

Plasma calcium concentrations tended to be low in the birds with the most advanced disease, but the lowest value was in a bird with a bone score of 3, and one bird with a bone score of 1 had a calcium concentration of 9.9 mg/dl. This is consistent with past observations that indicate that increased parathyroid activity, as long as there is bone to be reabsorbed, will

maintain calcium concentrations in the normal or sometimes increased range (Wallach and Flieg, 1970). The low plasma calcium in the bird with a bone score of 3 may have represented a transient drop in calcium, as expected in a bird in the early stages of secondary hyperparathyroidism.

The finding of salmonellosis in several egrets merits further investigation. These birds feed in the same fields as cattle and other livestock and often nest in suburban environments; therefore, they have the potential of being a human and animal health threat.

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