Osteodystrophy in Grey Parrots

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Internship report

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Abstract

Grey parrots are African parrots and they are the second most commonly traded psittacine birds in the world pet trade, desired mainly because of its excellent ability as a mimic. Hand reared grey parrot chicks often develop skeletal problems like osteodystrophy, similar to human rickets. Rickets is caused by a vitamin D deficiency, and because the symptoms of osteodystrophy and rickets are so similar it is believed that a vitamin D or calcium deficiency may be responsible for the skeletal deformities seen in hand-reared grey parrots. Others believe that the way in which the grey parrot chicks are fed and kept is also a contributing factor. This study tries to determine how much the nutritional and load-bearing factors are involved. Six high resolution $\mu$CT scans and 3 dimensional reconstructions where made, showing that these bone are normal and the amount of vitamin D and calcium in manmade parrot food is sufficient for adults. A histological study was done on hyacintr macaw embryos at hatching date, showing a partial calcification of the bones.
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1 Introduction

1.1 Parrots

In the warmer regions of the world parrots are a common site. Parrots can be found in India, Southeast Asia, southern regions of North America, South America and West Africa. By far the greatest number of parrot species comes from Australasia, South America, and Central America. There are about 330 different species of parrots. These 330 species of the family Psittacidae are divided into 7 subfamilies including the subfamily Psittacinae. This subfamily is divided into 5 tribes, one of which is the tribe Psittacini. This tribe contains 22 species of African parrots, including the grey parrot. The grey parrot (Psittacus e. erithacus) is widespread throughout equatorial Africa. It is the second most commonly traded psittacine bird in the world pet trade, desired mainly because of its excellent ability as a mimic. [1][2]

1.2 Altricial versus precocial

Parrots are altricial birds, unlike poultry, which are precocial birds. Altricial means that the newly born or hatched young are relatively immobile, have closed eyes, lack hair or down, and must be cared for by the adults. Precocial species are those that are relatively mature and mobile from the moment of birth or hatching. Precocial birds, including many ground-nesting species, have offspring that are born covered with down and ready to leave the nest in a short period of time following hatching (e.g. 24 hours). This amount of time is necessary because the offspring require time for their downy coats to dry prior to leaving the nest. Additionally, most precocial chicks lack thermoregulation (the ability to regulate their own body temperatures), and they depend on the attending parent(s) to provide an outside source of heat for a short period of time. Precocial birds find their own food. Examples of precocial birds include the domestic chicken, many species of ducks and geese, and rails and crakes. [1][3]

There are also 2 intermediated groups: semi-precocial and semi-altricial. Semi-precocial means that the chicks hatch with their eyes open, covered with down, and capable of leaving the nest soon after hatching (they can walk and often swim), but stay at the nest and are fed by parents. This developmental pattern is found in the young of gulls and terns. Semi-altricial birds are covered with down, incapable of departing from the nest, and fed by the parents. In species classified as semi-altricial 1, such as hawks and herons, chicks hatch with their eyes open. Owls, in the category semi-altricial 2, hatch with the eyes closed. [1]

Altricial nestlings grow rapidly, on average three to four times more quickly than precocial birds. Parrots are altricial birds and although they grow quickly compared with precocial birds, they are considered one of the slowest growing altricial birds. [1]

1.3 Skeleton

Because altricial birds stay in the nest their bones are only partially calcified when they hatch. The majority of the bone is still cartilage. [4]

The development of the embryonic skeleton in precocial and altricial birds has been studied by Blom and Lilja [5]. In all the species researched up to their study the skeletal elements ossify in identical sequence with little variation in the number of ossifications present at hatching. However, there is considerable variation between species in the degree of ossification at hatching. Therefore it has been suggested that there is a relationship between postnatal growth rate and degree of ossification at hatching.

Altricial birds have larger cartilaginous zones than precocial birds, which explains the faster growth rate of altricial birds compared with precocial species. The cartilage
volume of neonatal birds has been shown to vary from 50% in precocial birds to 90% in altricial species. The mechanical strength of the bones would be expected to be inversely proportional to the cartilage cell volume.

The skeleton has three main functions in young birds: to provide support for the musculature, and to resist the mechanical forces from both growth and locomotion. Although the skeleton of precocial hatchlings would be expected to provide all three functions, in altricial hatchlings the skeletons primary function would be support with limited locomotive forces. [4]

1.4 Parrots in captivity

The relationship between skeletal development and function in the parrot has been studied by Harcourt-Brown in 2004 [4]. The study examined the rate and cessation of growth of each long bone by radiography in naturally reared dusky parrots (Pionus fuscus). The pelvic limbs bones had finished growing by 39 days, the wing bones by 47 days. Observation of the behavior of the birds revealed no vigorous activity such as climbing or flapping until bone growth was complete. It was noted that an isolated bird would stand and walk around if given the opportunity. If the same bird was placed in contact with siblings it would huddle into them. This huddling behavior would help conserve body heat but it could also provide natural support to the growing bird. Any factor that might increase the locomotor activity of altricial birds could jeopardize normal bone development.

Captive breeding of the grey parrot has increased dramatically during the last decade as popularity of the species continues to increase. When these parrots are bred in captivity they are thought to be better pets because they are more attached to humans. [6]

According to Harcourt-Brown [6], 44% of all captive bred grey parrots develop skeletal problems. These problems vary from mild bending of the tibiotarsus (long bone in the leg of a bird, between the femur and the tarsometatarsus, consisting of the tibia fused with the proximal bones of the tarsus) to severe deformities in all limbs and the torso. The exact cause of these deformities is unknown. It is believed that it is caused by a nutritional problem combined with to-early load bearing of the limbs.

Parrots bred in captivity are frequently artificially reared by syringe or spoon-feeding. They are removed from the parents after hatching and kept individually in small plastic boxes without the support of their siblings. The birds are usually very mobile in these boxes, especially at feeding time. It has therefore been suggested that hand rearing altricial parrots might be a factor in the development of skeletal problems, like osteodystrophy, by removing the support of siblings combined with increased motor activity. [6]

1.5 Goal

The goal of this study is to determine if the nutritional and load-bearing factors are involved. First the nutritional problem will be explained. Then several techniques to determine the bone physiology of parrots are explained. Lastly an idea for future research will be given.
2 Nutritional problem

In humans the disease called rickets manifests the same symptoms as the skeletal problems and osteodystrophy seen in the hand reared grey parrots. Rickets is a softening of the bones in children, potentially leading to fractures and deformities, like the one shown in figure 1. [1]

Rickets is among the most frequent childhood diseases in many developing countries because the predominant cause is a vitamin D deficiency, but lack of adequate calcium in the diet may also lead to rickets. Although it can occur in adults (osteomalacia), the majority of cases occur in children suffering from severe malnutrition, usually resulting from famine or starvation during the early stages of childhood. Osteomalacia is the term used to describe a similar condition occurring in adults, generally due to a deficiency of vitamin D.

Figure 1: Left: Radiograph of a 2 year old rickets sufferer, with a marked genuvarum (bowing of the femurs) and decreased bone opacity, suggesting poor bone mineralization. Right: Ventrodorsal radiograph of a grey parrot, showing severe bowing of the tibiotarsus.

Because the symptoms of this disease are similar to the osteodystrophy seen in parrots, it has been hypothesized that a vitamin D or calcium deficiency may cause the skeletal deformities seen in hand reared parrots. Michael David Stanford [3] has studied the calcium metabolism of grey parrots. The following section gives a brief overview of his research, the entire study is provided as an attachment to this report.
2.1 Calcium metabolism in grey parrots: the effects of Husbandry

2.1.1 Control of calcium metabolism in birds

Calcium has two important physiological roles in the bird. It provides structural strength for the avian skeleton and has a vital role in many biochemical reactions within the body via its concentration as the ionized salt in tissue fluids. The control of calcium metabolism in birds has developed into a highly efficient homeostatic system able to respond quickly to sudden demands for calcium. This is required for the production of hard-shelled eggs and the rapid growth rate in young birds. Disorders of calcium metabolism are also common in captive grey parrots with signs ranging from osteodystrophy in young birds (due in part to the greater calcium requirement in young growing birds) to hypocalcaemic convulsions in adults. Although grey parrots are considered to be especially susceptible to disorders of calcium metabolism, problems have been reported in a variety of captive psittacine species.

2.1.2 Vitamin D$_3$ and calcium metabolism

The main role of vitamin D$_3$ is in the control of bone metabolism by both regulating mineral absorption and controlling the differentiation of its cellular elements. Vitamin D$_3$ (cholecalciferol) is the form of vitamin D found throughout the animal kingdom but only very rarely in plants. It is naturally found in fish, eggs, meat and milk. In plants, vitamin D occurs as vitamin D$_2$ (ergocalciferol), which most mammals can utilize as well as vitamin D$_3$. Birds cannot utilize vitamin D$_2$. This is due to increased renal clearance of vitamin D$_2$. In addition, the binding of vitamin D$_2$ to plasma proteins in birds is less efficient than in mammals. Birds acquire vitamin D$_3$ from a combination of endogenous synthesis and dietary supply.

The skin has been established as the organ for vitamin D$_3$ production in birds. Birds secrete 7-dehydrocholesterol onto featherless areas of skin. 7-dehydrocholesterol is converted to cholecalciferol by an ultraviolet (UV) light (wavelength of 285-315nm) dependent isomerization reaction.

The importance of the vitamin’s role in bone development and the requirement of ultraviolet light for its metabolism has been overcome by the commercial availability of dietary vitamin D$_3$ which is essential for the indoor production of poultry.

2.1.3 Metabolic functions of vitamin D in birds

Vitamin D is responsible for 70% of the calcium absorption in a growing chicken compared with 10% in a growing rat. Vitamin D$_3$ deficient animals have shorter intestinal villi, thereby reducing the surface area available for calcium absorption.

Vitamin D$_3$ has been shown to stimulate bone resorption indirectly by promoting osteoclast formation and activity. The process of bone calcification is related to the presence of cholecalciferol.

Vitamin D$_3$ is required for normal hatching of chicken eggs. Vitamin D$_3$ deficient chicken embryos are unable to complete the pre-hatching positional changes required for pulmonary respiration. In the same embryos, bone and muscle weights are lower than would be expected because of increased parathyroid gland activity.

2.1.4 The effects of ultraviolet radiation on vitamin D$_3$ metabolism

There are three UV wavelengths recognized: UVA (315-400 nm), UVB (290-315 nm) and UVC (100-280 nm). UVB radiation is associated with the photobiology of vitamin D$_3$. The UV light required for endogenous vitamin D$_3$ synthesis can either be supplied from full spectrum sunlight or using artificial lamps manufactured to provide UVB radiation.
Chickens exposed to 30 minutes of artificial UVB radiation whilst fed a vitamin D₃ deficient diet developed significantly less skeletal development problems than chickens denied supplementary UVB light.

2.1.5 Research

Michael Stanford’s research also involved a three year study of 40 healthy sexually mature grey parrots housed indoors as 20 pairs and their progeny. He studied the effects of diet and UV light and compared the results of his study group with blood samples of 20 captured wild grey parrots.

2.1.6 Conclusions

Michael D. Stanford concludes that nutritional secondary hyperparathyrothism is responsible for hypocalcaemia in adult grey parrots and for juvenile osteodystrophy in grey parrot chicks. He also concludes that increasing the dietary content of calcium and vitamin D₃ and/or providing artificial ultraviolet light in the 285-315 nm range significantly increases plasma ionized calcium and vitamin D₃ concentrations in grey parrots. Increasing these concentrations results in reduced signs of hypocalcaemia and osteodystrophy.
3 Materials and Methods

3.1 Available material

Because this study was done on naturally deceased parrots provided by The Dutch Research Institute for Birds and Uncommon Animals (NOIVBD), only a limited amount of material was available. The birds available for this study were: six grey parrots (Psittacus erithacus), varying in age between 111 days and 31 years, four hyacinth macaw (Anodorhynchus hyacinthinus) embryos at hatching date, one 15 day old green wing macaw (Ara chloroptera) and a 3 day old blue yellow macaw (Ara ararauna). All these animals where tame and most off them raised by their parents, and all ate pellets and other special parrot food for a large part of their lives. They all died of natural causes. All animals were necropsied and frozen by a veterinarian of the NOIVBD. Both hind limbs of the six grey parrots and the 15 day old green wing macaw where dissected and the left one was studied in a µCT to show the details of the bones and the trabecular structure. See figure 2 for a photo of the right legs of the parrots. The right leg of a hyacinth macaw embryo at hatching date was studied with a alcian blue and alizarin red staining, which results in a blue staining of the cartilage and a red staining of the calcified tissue. And from the hind limbs of another hyacinth macaw embryo at hatching date histological slides where made, with a safranin O and fast green staining, which results in a red staining of the cartilage and a dark blue staining of bone.

Figure 2: Photo of the right legs of the parrots. The most left, is the right leg of the embryo used for the alcian blue and alizarin red staining. The second from the left is of a 6 day old parrot. The third one from the left is the right tibiotarsus of the 15 day old green wing macaw. The 6 on the right are from the grey parrots used for the µCT images.

3.2 µCT

In order to determine the normal anatomy of a parrot tibiotarsus, µCT images are made from the legs of the grey parrots. High resolution scans where made and the tibiotarsus was reconstructed in 3D, see figure 3. For these reconstructions a fixed lower threshold value of 435 mg HA/ccm was used. This is an arbitrary value because the exact mineral content of grey parrot bone is unknown. These reconstructions were visually analyzed to determine the amount and pattern of the trabecular bone. Also a scan was made of the tibiotarsus of 15 day old green wing macaw.
3.3 Histology and staining

For both staining and histology the right hind limbs of two hyacinth macaw embryo’s at hatching date were used. Originally the staining and histology were planned as a method to determine the amount of calcified bone in grey parrot embryo’s and chicks, but because only hyacinth macaw embryo where available, the staining was used for a basic understanding of normal parrot-chick hind limb morphology.

3.3.1 Alcian blue and alizarin red staining

For the alcian blue and alizarin red staining a protocol normally used on chicken embryo’s was used. Because chickens are precocial birds and their legs are partially calcified at hatching, it was unclear how and if this staining would work for altricial birds. An alcian blue and alizarin red staining involves 2 staining steps and 2 KOH clearing steps. First the sample is placed in 95% ethanol, then the cartilage is stained with alcian blue. After 8 hours the sample is washed with 95% ethanol and placed in ethanol for 48 hours. The next step involves clearing the sample with 1% KOH for 3 hours or until it is sufficiently clear. Then an alizarin red staining of the calcified parts is done, followed by another clearing in 2% KOH. The sample is fixed in 4% PFA and stored in PBS. For this study the hind limb of a hyacinth macaw embryo at hatching date was dissected and not the entire embryo was stained.

3.3.2 Safranin O and fast green staining

The hind limb of a hyacinth macaw embryo at hatching date was dissected, placed in a tissue processor, imbedded in wax and sectioned using a microtome. The sections where placed on microscope slides and colored using a safranin O and fast green staining protocol resulting in red stained cartilage and blue bone. The slides where analyzed using a light microscope.
4 Results

4.1 µCT

The reconstruction showed that the 3 youngest parrots of 111, 156 and 161 days have mature bone, but the trabecular bone is not as highly structured as in the older parrots, see figure 4 and 5. The bone of adult parrot is very structured. Also the older parrots seem to have a natural bending of their legs. Also a scan was made of the tibiotarsus of 15 day old green wing macaw. The scan showed that this bone was largely made of cartilage, see figure 6. The 3D reconstruction showed that only the center of the bone was calcified and the ends were still cartilage. The lower threshold value had to be lowered to 300 mg HA/ccm to obtain useful results. It is not clear if this lower mineral content is only caused by the age of animal or also because it is a different species of parrot.

![Figure 4: Close up of the 3D reconstruction of the tibiotarsus of a 111 days old grey parrot. The trabecular bone is clearly visible.](image)

4.1.1 Parrot with one crooked leg

When preparing the parrots for the µCT scanner, one parrot appeared to have one crooked leg while the other one was completely normal. This deformity was only visible after dissection of the leg. Figure 7 is the scan view roentgen photo of this crooked leg, with a transversal slice through the bend. The leg bent outward, toward the fibula. Using the µCT images a support like structure is seen at the beginning of the bend.

4.2 Histology and staining

Figure 8 shows one of the histological slides colored with safranin O and fast green. The slides showed that the central part of the bone is calcified and the ends are still cartilage. The slides where difficult to make because of the bone fragments present in the sample.

A visual inspection of the limb stained with alcian blue and alizarin red showed again that the ends of the limb where cartilage and the central region was bone. No foto of the
Figure 5: Close up of the 3D reconstruction of the tibiotarsus of a 31 year old grey parrot. The highly structured trabecular bone is clearly visible. Bird bones are hollow to reduce weight for flying.

limb stained with alcian blue and alizarin red is included because the image quality of the photo’s was not good enough to see the differences between bone and cartilage.
Figure 6: 3D reconstruction of the tibiotarsus of a 15 day old green wing macaw. The ends are missing because they are still cartilage.

Figure 7: Scanview of the leg of a 6 year old parrot, and a transversal slice through the bend.
Figure 8: Photo's of slides made from a hind limb of a hyacinth macaw embryo at hatching date. Cartilage is red, bone is dark blue. The cartilage is present at the ends of the bone and not in the middle, indicating that the bone is partially calcified.
5 Discussion

Michael David Stanford showed in his research that the amount of vitamin D in the diet of parrots is a very important factor for the normal development of young parrots. But what remains unclear in his study is the influence of loading on the extremities.

The results from the \( \mu \)CT and the histology and staining show that adult parrots have normal bone and embryos and hatchlings have only partially calcified bone. The differences between cartilage and bone where difficult to see in the limb stained with alcan blue and alizarin red, because the alizarin red coloring was not dark enough. The protocol for the alcan blue and alizarin red staining was originally designed for chicken embryo’s and strictly followed for this study. The tint of the red coloring was not as expected and it is not clear if the physiology of a parrot bone, or the time taking to color the limb is the cause of the light red coloring.

The roentgen and \( \mu \)CT images of the parrot with one crooked leg show that normal internal bone support structures are build. The leg of this parrot bent outward, toward the fibula. In osteodystrophy or hypocalcemia the legs tend to bend inward, although in extreme cases all directions are possible. This means that the bone physiology is completely normal in adult domestic parrots, showing that their diet contains enough vitamin D and calcium.

Michael D. Stanford \[3\] also showed that growing chicks need substantial amounts of calcium and vitamin D. A way to determine if the manmade parrot food contains enough calcium and vitamin D for growing chicks is by looking at the calcification speed of the bones. Parrots are altricial birds. This means that when they hatch they stay in the nest and their bones are not yet fully calcified. The calcification of the bones is a gradual process. It is possible to determine something like a calcification speed, the amount of mineralized bones after a given amount of days. This can be done with the help of roentgen photos, as shown by Harcourt-Brown \[4\]. The rate of calcification will slow down when a vitamin D or calcium deficiency is present \[3\]. If the calcification speed slows down, the bone might not be fully calcified when it has to start bearing weight. This could result in bent legs. If to early weight bearing causes the leg bending, the calcification speed of the bones of hand reared parrots will be the same as the calcification speed of the bones of naturally raised, wild parrots. A comparison between hand raised and naturally raised parrots will be necessary. Because grey parrots are an endangered species, no chicks can be sacrificed. A way to determine if there is a difference in the calcification speed between naturally and hand raised parrots, could be by taking roentgen photos of wild and hand raised grey parrots chicks every couple of days and comparing the calcification speeds.

CT images would be even better, but birds are difficult to anesthetize and the risks of fatalities would be to great.

To determine if the osteodystrophy seen in hand-reared chicks is caused by the way they are kept, the conditions should be equalized with the normal condition in the nest. Something like a small basket or a hammock with holes for their legs could be created, to simulate the support they normally get from their siblings.

6 Conclusions

The full grown, tame grey parrots used for this have normal bones, showing that their diet contained enough vitamin D and calcium. Hyacint macaw embryo’s have only partially calcified bone.

\( \mu \)CT, roentgen and alcan blue and alizarin red staining are very useful techniques to determine the amount of calcification and the physiology of a parrot leg. \( \mu \)CT and roentgen are very usable with full grown parrots, and alcan blue and alizarin red staining with un-full grown. Using normal slides and safranin O and fast green staining might
work on really young chicks and embryos if the amount of calcification in the bones is sufficiently low enough.
References