Bone composition and bone mineral density of long bones of free-living raptors

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Abstract

Bone composition and bone mineral density (BMD) of long bones of two raptor and one owl species were assessed. Right humerus and tibiotarsus of 40 common buzzards, 13 white-tailed sea eagles and 9 barn owls were analyzed. Statistical analysis was performed for influence of species, age, gender and nutritional status. The BMD ranged from 1.8 g/cm^3 (common buzzards) to 2.0 g/cm^3 (white-tailed sea eagles). Dry matter was 87.0% (buzzards) to 89.5% (sea eagles). Percentage of bone ash was lower in sea eagles than in buzzards and owls. Content of crude fat was lower than 2% of the dry matter in all bones. In humeri lower calcium values (220 g/kg fat free dry matter) were detected in sea eagles than in barn owls (246 g/kg), in tibiotarsi no species differences were observed. Phosphorus levels were lowest in sea eagles (humeri 104 g/kg fat free dry matter, tibiotarsi 102 g/kg) and highest in barn owls. Calcium-phosphorus ratio was about 2:1 in all species. Magnesium content was lower in sea eagles (humeri 2590 mg/kg fat free dry matter, tibiotarsi 2510 mg/kg) than in buzzards and owls. Bones of barn owls contained more copper (humeri 8.7 mg/kg fat free dry matter, tibiotarsi 12.7 mg/kg) than in the Accipitridae. Zinc content was highest in sea eagles (humeri 278 mg/kg fat free dry matter, tibiotarsi 273 mg/kg) and lowest in barn owls (humeri 185 mg/kg, tibiotarsi 199 mg/kg). The present study shows that bone characteristics can be considered as species specific in raptors.

Introduction

Raptors, or birds of prey, are a heterogeneous group of birds. Most prey on other animals, have keen eyesight, curved beaks and powerful talons. Today, raptors are splitted taxonomically in two different orders: the falconiformes and the accipitriformes. Common buzzards and white-tailed sea eagles are classified in the family Accipitridae.[1] Common buzzards occur widely in nearly the whole of Europe and parts of Asia. They live in habitats which combine woodland for nesting and open landscape for hunting, and prey mostly on small mammals.[2] White-tailed sea eagles can be found in central and northern Europe and large parts of northern Asia in wetland habitats with big trees and on coasts. They are feeding mostly on fish and waterfowl.[2] Barn owls belong to the family Tytonidae in the order Strigiformes. Their area covers Europe, parts of Asia, Australia and Africa besides deserts and most of America. They prefer grassland habitat with a high density of small rodents, especially voles, and use nesting sites in buildings, caves and trees.[3] Bone composition varies between different species and depends on multiple factors like gender, age, breed, feed and habitat.[4,5] For raptors only a single publication exists, based on an individual falcon and a single buzzard without information on species, origin, gender, age or feeding.[6]

The bone tissue includes inorganic and organic components. The dry matter in broiler bones is 45% to 88%,[7] 87% in turkey bones and increases with age.[8] Examining a single buzzard and a single falcon WEISKE shows a dry matter of 77.2% and 74.4% in the whole skeleton.[6] In chicken the content of bone ash in dry matter is 47.7% to 70.3%.[4,9-13] The bone ash in turkeys[8,14] and geese[13] is around 70%. Analyzing the whole skeleton of different psittacines reveals bone ash of 58.2% to 59.8%, while canaries have a higher content of 62%.[15] The only values available for raptors are bone ash contents of 63.6% in a single buzzard and 64.1% in a single falcon.[6] Bone ash is higher in female birds[12] and increases with age.[16]

In poultry the content of crude fat is 26-31% of the dry matter,[10] in a buzzard 16.7% and in a falcon 17.7%.[6] Contrary to mammals the content of crude fat remains more or less constant during growth in chicken.[5]

The skeleton contains over 99% of the calcium and about 80% of the phosphorus in the body[17] in a ratio of 2:1.[13] A calcium content of 200 g/kg to 212 g/kg fat free dry matter and a phosphorus content of 102 g/kg to 108 g/kg fat free dry matter is found in the bones of different pet bird species. This is consistent with a ratio of 1.9:1 in all species beside of budgerigars that show a ratio of 2.5:1.[15] The long bones of poultry (chicken, turkeys and geese) have similar contents with 215 g/kg to 354 g/kg calcium and 104 g/kg to 167 g/kg phosphorus.[8,10,13,14] The analysis of the skeleton of a single buzzard reveals a calcium content of 325 g/kg ash, the one of a single falcon of 329 g/kg ash.[6] The calcium content of the bones increases with age.[5] The results for the phosphorus content are inconsistent.[8,18] The bones of female chicken have lower levels of calcium and phosphorus than the bones of male individuals.[19] In poultry there seems to be no major variation of calcium and phosphorus between the different bones.[5,18]

From the total magnesium, 60% are located in the bones with a third embedded in hydroxyapatite.[17] The bones of psittacines, canaries, chicken, geese and 46 different species of wild ranging birds have magnesium concentrations from 2300 mg/kg to 4181 mg/kg.[13,15,19] A comparable content is found in a single falcon with 4700 mg/kg ash magnesium, while a single buzzard has slightly higher values with 6100 mg/kg ash.[6] The magnesium content of the bones decline marginally with age.[18] Male chicken have lower contents than females.[19]

Copper is an important cofactor for the formation of collagen crosslinks.[20] Most of the copper is bond to the organic matter of the bone.[21] The copper content of chicken bones varies in literature from 2.54 mg/kg to 10.0 mg/kg fat free dry matter.[10,21-23] The skeleton of different psittacine species contains 8.1 mg/kg to 9.1 mg/kg fat free dry matter copper while

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budgerigars have slightly higher contents of 11.5 mg/kg.[15] The bones of day-old chicks have higher copper contents than the bones of three week old chicks.[21] Gender has no influence on the copper content of bones.[19,21]

Zinc is an enzyme cofactor that influences bone growth.[17] Zinc contents in poultry bones vary from 122 mg/kg to 212 mg/kg.[10,14,19] The skeleton of psittacines contains comparable amounts of copper with 205 mg/kg to 298 mg/kg fat free dry matter, while canaries have higher values of 696 mg/kg.[15] No data could be found on the influence of age on the zinc content of bones. The femora of male chicken have a lower zinc content than in females.[19]

The mass density of bones is also called BMD (bone mineral density). BMD is influenced by age, gender, feed and growth rate. With increasing age BMD ascends.[12,16] Female birds have a higher BMD than males.[12,24] Breeds with a slower growth rate show a higher BMD.[16] The calcium and phosphorus content of the feed influences the BMD.[25,26] The three-dimensional structure of the bone also determines its BMD.[27] Dry matter, bone ash, mineral content and mechanical properties of a bone correlate with the BMD.[28,29] For assessing BMD different methods are used, whose results are not always comparable.[27,30,31] The classical method using Archimedes principle correlates with the results of Dual-energy X-ray absorptiometry (DXA) and Peripheral quantitative computed tomography (pQCT).[11,31,32] In contrast to the classical method DXA and pQCT are non-invasive and can be used on live animals.[31,33] DXA only provides the amount of absorbed beams by the bone, while pQCT is able to measure the real physical volumetric density of the bone that is achieved with the classical method.[31,34] The BMD of bird bones using Archimedes principle or pQCT is quoted from 0.36 g/cm³ to 1.79 g/cm³.[11,12,16,24,28,30,35]

The goal of this study was to assess the bone composition and the bone mineral density of the long bones of two raptor and one owl species to evaluate similarities and differences compared to better studied bird species.

Materials and Methods

The right humerus and the right tibiotarsus of 53 raptors [common buzzard (*Buteo buteo*) n=20 ♂, n=20 ♀, white-tailed sea eagle (*Haliaeetus albicilla*) n=7 ♂, n=6 ♀] and nine barn owls (*Tyto alba*) [n=4 ♂, n=5 ♀] that died or were euthanized at the small animal hospital of the Freie Universität Berlin were examined. To obtain adequate numbers of white-tailed sea eagles and barn owls bones from animals found dead in the wild were included in the study.

The age of the common buzzards and the white-tailed sea eagles was determined by means of feather characteristics, beak and iris color according to Forsman.[36] After finishing the feather growth a definite age determination in barn owls is not possible. All owls that could not be identified as hatchlings or fledglings were considered to be adults.[37] In common buzzards the same number of juveniles and adults was included in the study. The gender was determined post mortally by visual examination of the gonads. In all species the gender distribution was balanced. The nutritional status of every animal was evaluated and data considering illnesses and time in captivity were collected.

The bones were extracted without damaging the surface, the soft tissue was removed manually and the bones were stored in air-proof plastic bags at –20°C. For the following analyses the bones were wrapped in moist gauze and thawed for 12 hours at room temperature.

Bone mineral density

Bone mineral density was analyzed using the classical method following Archimedes principle. A randomly chosen bone piece of the diaphysis was weighed on a precision balance (Sartorius 2024 MP, Sartorius AG, Göttingen, Germany). The first value was obtained normally (m_{Air}), the second was obtained under water (m_{Water}). The difference ($m_{Air} - m_{Water}$) resembles the buoyant force of the bone piece. Regarding the temperature dependent density of water the temperature was evaluated at each weighing and the corresponding water density included in the formula.[38] The physical density was calculated using the following formula:

$$\rho = \frac{m_{Air}}{m_{Air} - m_{Water}} \cdot \rho_{Water}$$

Bone composition

After breaking them up the bone diaphyses of common buzzards and barn owls were milled with a swing mill (MM200, Retsch GmbH, Haan, Germany). The bones of the white-tailed sea eagles were milled with an electric mill (no manufacturer data).

All tests for analyzing bone composition were accomplished in accordance with standard methods.[39] Dry matter was analyzed using a heating cabinet with 103°C until weight constancy (Heraeus ST5042, Heraeus Holding, Hanau, Germany). Crude fat was determined randomly using a Soxhlet extractor (Extraction System B-811, Büchi Labortechnik AG, Flawil, Switzerland). Bone ash was obtained after ashing in a muffle furnace at 550°C (Heraeus thermiconP, Heraeus Holding, Hanau, Germany). The phosphorus content was determined using the vanadate-molybdate-method (Ultrospec 2000, Pharmacia Biotech, Vienna, Austria). Calcium, magnesium, copper and zinc were analyzed with atomic absorption spectroscopy (AAS vario 6, Analytik Jena, Jena, Germany).

All data were analyzed with SPSS 15.0 (SPSS, Chicago, IL, USA). Differences and their significance between species, gender and age were tested with Mann-Whitney-U test. Significance of differences between humeri and tibiotarsi were checked with the Wilcoxon test (significant when $P<0.05$). Correlations of the different parameters were evaluated by Spearman's rank correlation coefficient (significant when $r_S>0.25$).

Results

The median, minimum and maximum values for all parameters evaluated in the three species are displayed in Tables 1-3.

Dry matter of the humeri and the tibiotarsi was lower in juvenile buzzards compared to adults (humerus P=0.001, tibiotarsus P=0.004). None of the species showed a gender difference (humerus buzzards P=0.829; eagles P=1.0; owls P=0.730; tibiotarsus buzzards P=0.262; eagles P=0.429; owls P=0.905). Positive correlations of the dry matter of humeri and tibiotarsi were detected for all three species (buzzards r_S=0.448, P=0.004; eagles r_S=0.759, P=0.003; owls r_S=0.983, P<0.001). Crude fat was tested randomly in eleven humeri and eight tibiotarsi of different animals. The crude fat content was lower than 2% in all bones (median=0.28 %). Bone ash was lower in the humeri and tibiotarsi of juvenile buzzards compared to adults (each with P<0.001). No gender difference was found for ash content in humeri or tibiotarsi (humerus buzzards P=0.626; eagles P=0.429; owls P=0.556; tibiotarsus buzzards P=0.358; eagles P=1.000; owls P=0.413). Bone ash of humeri and tibiotarsi correlated positively in common buzzards (r_S=0.802, P<0.001).

Lower calcium content was found in humeri and tibiotarsi of juvenile buzzards compared to adults (each with P<0.001). Humeri of male barn owls displayed lower values than the humeri of females (P=0.016). None of the species showed a gender difference in the calcium content of tibiotarsi (buzzards P=0.914; eagles P=0.247; owls P=0.086). Calcium content of humeri and tibiotarsi correlated positively in common buzzards and barn owls (buzzards r_S=0.343, P<0.001; owls r_S=0.712, P=0.031). Humeri and tibiotarsi of juvenile common buzzards displayed less phosphorus than in adults (each with P<0.001). No gender difference was found in humeri (buzzards P=0.646; eagles P=0.931; owls P=0.905). In barn owls males had lower phosphorus content in tibiotarsi than females (P=0.05). Phosphorus content of humeri and tibiotarsi correlated positively in common buzzards (r_S=0.832, P<0.001). The median calcium/phosphorus ratio of the humeri was 2.1:1 in all three species. In the tibiotarsi common buzzards had a ratio of 2.1:1, white-tailed sea eagles of 2.3:1 and barn owls of 2.0:1. Humeri of juvenile common buzzards contained more magnesium than in adults (P<0.001). No difference was found for juveniles and adults in the tibiotarsi (P=0.060). No gender difference existed in the humeri (buzzards P=0.213; eagles P=0,792; owls P=0.111), while tibiotarsi of male common buzzards had lower magnesium content than in females (P=0.021). The magnesium contents of humeri and tibiotarsi showed a positive correlation in common buzzards (r_S=0.568, P<0.001) and barn owls (r_S=0.706, P=0.034). In common buzzards no difference existed for the copper concentration in humeri and tibiotarsi in juveniles and adults (humerus P=0.695, tibiotarsus P=0.839). None of the species showed a gender difference (humerus buzzards P=0.978; eagles P=1.000; owls P=0.730; tibiotarsus buzzards P=0.229; eagles P=0.931; owls P=0.730). The copper content of humeri and tibiotarsi correlated positively in common buzzards (r_S=0.328, P=0.039). No age (humerus P=0.372, tibiotarsus P=0.745) or gender difference could be found for the zinc content in humeri and tibiotarsi (humerus buzzards P=0.194; eagles P=0.247; owls P=0.413; tibiotarsus buzzards P=0.185; eagles P=0.792; owls P=0.296). Zinc content of humeri and tibiotarsi correlated positively in common buzzards (r_S=0.594, P<0.001) and barn owls (r_S=0.904, P=0.001).

In common buzzards juveniles displayed lower BMD than adults (humerus P=0.025, tibiotarsus P=0.037). None of the species showed a gender difference (humerus buzzards P=0.745; eagles P=0.429; owls P=0.730; tibiotarsus buzzards P=0.291; eagles P=0.792; owls P=0.413). No correlation existed for the BMD of humeri and tibiotarsi (buzzards r_S=0.883, P=0.883; eagles r_S=0.394, P=0.183; owls r_S=-0.008, P=0.983).

Discussion

Evaluating the results of the present study has the confinement of using animals that died or were euthanized due to severe injuries. The health status of the birds might have influenced the bone characteristics, however, due to the high number of animals, it can be expected that the data are more or less representative. To evaluate the impact of the health status on the different bone parameters the birds' nutritional status was analyzed.

Data on raptors is limited in literature and no information is available if there are any differences in bone characteristics between free-living animals and those in captivity. But as habitat and feed have significant influence on bone composition,[5] this is very likely. This assumption leads to possible ecological implications as human-made changes of habitat and food sources could lead to pathological changes in bone characteristics.

Dry matter of the bones of the three species in this study was much higher than values for

Table 1. Median, minimum and maximum values for the bone composition in the humeri of the three different raptor species.

	Common buzzards			White-tailed sea eagles			Barn owls		
	Median	Min	Max	Median	Min	Max	Median	Min	Max
Dry matter, %	87.6	80.5	91.2	89.5	85.9	90.1	88.3	87.5	90.8
Bone ash, % dry matter	64.7	57.6	68.2	61.1	56.0	62.6	67.2	65.1	69.3
Crude fat, %	0.3	0.2	1.4	0.2	0.02	0.3	1.4	1.0	1.7
Calcium, g/kg fat free dry matter	225	185	274	220	180	240	246	204	253
Phosphorus, g/kg fat free dry matter	112	93.3	123	104	94.7	109	118	112	123
Magnesium, mg/kg fat free dry matter	3485	2690	4720	2590	2150	3660	3640	3180	3810
Copper, mg/kg fat free dry matter	6.14	4.88	17.1	5.76	4.55	16.6	8.65	7.05	27.5
Zinc, mg/kg fat free dry matter	207	166	278	278	193	295	185	156	216

Table 2. Median, minimum and maximum values for the bone composition in the tibiotarsi of the three different raptor species.

	Common buzzards			White-tailed sea eagles			Barn owls		
	Median	Min	Max	Median	Min	Max	Median	Min	Max
Dry matter, %	87.0	76.7	89.6	88.4	82.3	89.9	88.3	87.5	90.6
Bone ash, % dry matter	64.9	58.4	69.9	59.7	56.9	61.9	65.3	62.4	67.8
Crude fat, %	0.3	0.2	0.9	0.6	0.03	1.2	0.7	0.06	1.3
Calcium, g/kg fat free dry matter	235	202	262	230	208	248	239	221	250
Phosphorus, g/kg fat free dry matter	110	101	118	102	93.2	109	116	112	120
Magnesium, mg/kg fat free dry matter	3395	2730	3980	2510	2230	2830	3720	3210	4150
Copper, mg/kg fat free dry matter	5.85	4.13	8.88	5.28	4.44	15.0	12.7	7.09	19.9
Zinc, mg/kg fat free dry matter	219	182	290	273	201	287	199	164	215

Table 3. Median, minimum and maximum values for the bone mineral density (BMD) in the 3 different raptor species.

	Common buzzards			White-tailed sea eagles			Barn owls		
	Median	Min	Max	Median	Min	Max	Median	Min	Max
BMD humeri, g/cm³	1.91	1.49	2.11	1.98	1.79	2.02	2.00	1.90	2.15
BMD tibiotarsi, g/cm³	1.80	1.22	2.12	1.99	1.76	2.04	1.91	1.64	2.05

broiler chicken.[7] Although juvenile common buzzards had lower contents of dry matter than adults, their dry matter content was still much higher than in the broiler chicks. The dry matter of a mixed bone sample of a single falcon and a single buzzard where nearly has high as the values in the present study.[6] The bones of raptors therefore contain less liquid than the bones of broilers. If this fact is based on feeding, husbandry or the young age of the broiler chicks is not clear.

As in broilers and turkeys,[7,8] dry matter of the bones of juvenile common buzzards was lower than in adults. During growth bones are mineralized,[40] so mineral content[5,8] and consequently the dry matter increase.[41]

According to Rath *et al*.[12] bone ash of different bird species is comparable. Although bone ash of sea eagles was lower than in buzzards and owls, all ash contents in the present study were similar to other bird species.[6,11,14,15] Like in chicken bones bone ash was higher in adult common buzzards than in juveniles.[16] In contrast to chicken where female animals have a higher bone ash there was no sex difference in the raptors.[12] As the bone ash content depends on the mineral content,[41] bones of laying hens might contain more bone ash. Rath *et al*.[12] gave no information on the reproductive state of their animals but using animals from a commercial farm and the existence of medullary bone make this shortcut presumably. With less than 2% in all bones examined, the fat content was lower than values for poultry with 26% to 31%,[10] and raptors with 17% in a buzzard and 18% in a falcon.[6] The content in owl bones was higher with 1% of the dry matter compared to the bones of buzzards and eagles with 0.3% und 0.2%. The fat content of bones depends on individual variations and on feeding. The aliment of poultry and raptors is so different, that it can explain these differences. The raptors, in Weiskes study,[6] might have been birds from captivity so that the difference may be caused by this. In the present study bones were cleaned before analyzing the content, whereas in other studies the whole skeletons were used. The bone marrow has a high fat content so this influences the results as well. As the fat content was so low in the 19 bones that were examined, this fact was disregarded in the following tests as only a small incertitude would result compared to the analysis error.

The calcium content of humeri and tibiotarsi was 204 g/kg to 260 g/kg fat free dry matter which is comparable to the results in cage birds with 200 g/kg to 221 g/kg fat free dry matter,[15] wild birds with 229 g/kg bone,[42] turkeys with 237 g/kg dry matter,[8] chicken with 236 g/kg and geese with 269 g/kg fat free dry matter.[13] Like in chicken,[5] the calcium content of the bones in common buzzards was lower in juveniles than in adults. This is the result of progressive mineralization during growth and aging.[5,40] In this study no gender difference was found for the calcium content in common buzzards and white-tailed sea eagles. In contrast Vo *et al*.[19] found less calcium in the femur of adult male chicken compared to females. This was also the case in the humeri of barn owls in this study. The higher content of calcium in females could be associated with the reproductive status, as they store calcium in their bones during the egg laying period.[43] In the free living barn owls this could not be evaluated as no history on the birds existed, but all birds where found outside the normal reproductive time of this species and no medullary bone was visible.

The phosphorus content had differences in the three species in this study. The median of 103 g/kg to 118 g/kg fat free dry matter in the humeri and 101 g/kg to 117 g/kg fat free dry matter in the tibiotarsi still matches the data of other bird species like cage bird with 102 g/kg to 108 g/kg fat free dry matter,[15] wild birds with 110 g/kg bone[42] and poultry with 104 to 167 g/kg.[8,10,13,14] Like in turkeys the phosphorus content in juveniles was lower than in adults.[8] The reason for this is the maturation of the skeleton.[40] The tibiotarsi of male barn owls had a lower content of phosphorus than in females. Vo *et al*.[19] found the same effect in chicken. In contrast to poultry, where the phosphorus content does not vary in the different bones,[5,18] the humeri of common buzzards and barn owls had higher contents compared to the tibiotarsi. According to Field *et al.*[5] the phosphorus content of different bones is highly variable in mammals compared to poultry. This effect in raptors could be a species specific variation.

The average ratio of calcium and phosphorus is 2:1 in all animals.[13] In cage birds it was 1.9:1,[15] while the median ratio in raptors in this study was 2:1 to 2.3:1.

The magnesium content in bone ash is similar in most vertebrates but very variable in birds.[13] Existing data shows a magnesium content of 2550 mg/kg to 3920 mg/kg fat free dry matter in cage birds,[15] of 3600 mg/kg bone in wild birds[42] and 2300 mg/kg to 4181 mg/kg in different poultry species.[10,13,19] The magnesium content in the skeleton of a single falcon was 4700 mg/kg fat free dry matter.[6] The results of the present study with a magnesium content of 2435 to 3745 mg/kg fat free dry matter are comparable to the cited data. In poultry the magnesium content of bones decreases slightly with increasing age.[18] The humeri of juvenile common buzzards displayed higher content of magnesium than in adults, while this difference was not detectable in the tibiotarsi. Like in chicken the tibiotarsi of male common buzzards had a lower magnesium content than in females.[19] This gender difference was not existent in white-tailed sea eagles and barn owls. According to Vo *et al*.[19] the higher mineral content is the result of the egg laying process in female chicken. Compared to chicken the reproductive activity of raptors is not as intense why the mineral content might not display as marked gender differences. Taylor *et al*.[18] found similar contents of magnesium in the different bones of one bird. This was also the case for white-tailed sea eagles while small differences between the humeri and tibiotarsi where found in common buzzards and barn owls. As only a third of the magnesium in the bone is bond firmly to the apatite structure bone remodeling or fluctuations of the magnesium level in the blood can influence the actual magnesium content in the bone ash.[17]

Despite variation of the copper content in the bone ash of buzzards, eagles and owls the median content of 5.3 to 17.2 mg/kg fat free dry matter reflects the results in literature with 8.1 mg/kg to 9.4 mg/kg fat free dry matter in cage birds[15] and 2.5 mg/kg to 10.0 mg/kg fat free dry matter in chicken.[10,21,23] According to Doyle[44] the copper content of bone varies highly depending on the age and decreases according to Hill with increasing age.[21] This was not the case in common buzzards where no age difference was found. In the study of Hill animals in an age of one day and three weeks were used without comparing the results to adult animals.[21] Like in chicken no raptor species displayed gender differences in the copper content.[19,21]

The zinc content in chicken bones varies widely from 25.5 mg/kg dry matter[45] to 713 mg/kg dry matter.[22] In psittacines it was 205 to 298 mg/kg fat free dry matter[15] and 184 mg/kg ash in turkeys.[14] Although the species in this study showed differences in the zinc content the median results of 170 mg/kg to 278 mg/kg fat free dry matter are in the range of the values in literature. This variability is explainable with different feed and interspecies differences.[22,46] Like in humans no gender or sex differences where found.[47] The higher zinc content in the bones of female chicken is supposed to be related to the reproductive activity.[19]

The BMD was measured by the classical method using Archimedes principle because the obtained data is physically very exact. Although this method is only implementable to isolated bones of dead specimens the advantages of this method were predominant, as the results are comparable to modern, non invasive techniques like DXA and pQCT.[11,31,32] Like this the results of future studies on live animals with pQCT can be related to the results of this study. The BMD was lower in buzzards than in eagles and owls. The BMD of the humeri of common buzzards was also lower than in barn owls. For budgerigars a BMD of 0.36 g/cm^3 was evaluated,[24] the BMD of poultry was between 1.1 and 1.8 g/cm^3.[11,12,16,28,30,35,48] The median values of 1.69 bis 2.01 g/cm^3 in this study are at the higher end of these values respectively a little higher. Raptors have very solid and loadable bones, what might be the result of the high BMD.[16] Like in chicken the BMD was higher in adult common buzzards than in juveniles.[12,16] In contrast to chicken and budgerigars no sex difference was found in the three raptor species.[12,24] The budgerigars were examined during the egg laying period, for the chicken only the age of 72 weeks was specified. During the reproductive period more minerals are stored in the bones,[43] what increases the BMD.[32] The females in this study except one common buzzard were all examined outside their reproductive period what could explain the lack of a sex difference.

It was the aim of the present study to evaluate the bone composition and bone density of the long bones of raptors. Species differed distinctively and this needs to be considered for diagnostic purposes and bone physiology in raptors.

References

1. International Ornithologist's Union. IOC World Bird List. Available from: http://www.worldbirdnames.org. Accessed on: September 2014.
2. Glutz von Blotzheim U, Bauer K, Bezzel E. Handbuch der Vögel Mitteleuropas. Band 4: Falconiformes. 2. Aufl. Wiesbaden: Aula-Verlag; 1989.
3. Glutz von Blotzheim U, Bauer K. Handbuch der Vögel Mitteleuropas. Band 9: Columbiformes - Piciformes. 2. Aufl. Wiesbaden: Aula-Verlag; 1994.
4. Aerssens J, Boonen S, Lowet G, et al. Interspecies differences in bone composition, density, and quality: potential implications for in vivo bone research. Endocrinology 1998;139:663-70.
5. Field RA, Riley ML, Mello FC, et al. Bone composition in cattle, pigs, sheep and poultry. J Anim Sci 1974;39:493-9.
6. Weiske H. Untersuchungen über Qualität und Quantität der Vogel-Knochen und Federn in verschiedenen Altersstadien. Landwirtschaftliche Versuchs-Stationen 1889;36:81-103.
7. Yalcin S, Ozkan S, Coskuner E, et al. Effects of strain, maternal age and sex on morphological characteristics and composition of tibial bone in broilers. Br Poult Sci 2001;42:184-90.
8. Korfmann MA. Zur Skelettentwicklung und Wachstumsdynamik der Beckengliedmaße bei Mastputern (makroskopische, mikroskopische, radiologische, osteodensitometrische und mineralstoffanalytische Verlaufsuntersuchungen). Thesis Dissertation. Berlin: University of Berlin; 2003.
9. Thorp BH, Waddington D. Relationships between the bone pathologies, ash and mineral content of long bones in 35-day-old broiler chickens. Res Vet Sci 1997;62:67-73.
10. Rodenhoff G, Bronsch K. Die Zusammensetzung der Humerus-Kompakta bei Mastgeflügel mit und ohne Skelettanomalien. Zentralbl Veterinarmed A 1971;18:234-42.
11. Kim WK, Ford BC, Mitchell AD, et al. Comparative assessment of bone among wild-type, restricted ovulator and out-of-production hens. Br Poult Sci 2004;45:463-70.
12. Rath NC, Balog JM, Huff WE, et al. Comparative differences in the composition and biomechanical properties of tibiae of seven- and seventy-two-week-old male and female broiler breeder chickens. Poult Sci 1999;78:1232-9.
13. Biltz RM, Pellegrino ED. The chemical anatomy of bone. I. A comparative study of bone composition in sixteen vertebrates. J Bone Joint Surg Am 1969;51:456-66.
14. Crespo R, Stover SM, Shivaprasad HL, et al. Microstructure and mineral content of femora in male turkeys with and without fractures. Poult Sci 2002;81:1184-90.
15. Rabehl N. Untersuchungen zur Körperzusammensetzung und deren Entwicklung bei verschiedenen Ziervogelarten (Kanarien, Wellensittiche, Agaporniden, Nymphensittiche, Amazonen und Graupapageien) Thesis Dissertation. Hannover: University of Hannover; 1995.
16. Leterrier C, Nys Y. Composition, cortical structure and mechanical properties of chicken tibiotarsi: effect of growth rate. Br Poult Sci 1992;33:925-39.
17. Dressler D. Mineralische Elemente in der Tierernährung. Stuttgart: Eugen Ulmer; 1971.
18. Taylor TG, Moore JH, Hertelendy F. Variations in the mineral composition of individual bones of the skeleton of the domestic fowl. Br J Nutr 1960;14:49-57.
19. Vo KV, Boone MA, Torrence AK. Electrolyte content of blood and bone in chickens subjected to heat stress. Poult Sci 1978;57:542-4.
20. Rucker RB, Parker HE, Rogler JC. The effects of copper on collagen cross-linking. Biochem Biophys Res Commun 1969;34:28-33.
21. Hill R. The manganese and copper of chick bone. Br J Nutr 1965;19:163-70.
22. Southern LL, Baker DH. Zinc toxicity, zinc deficiency and zinc-copper interrelationship in Eimeria acervulina-infected chicks. J Nutr 1983;113:688-96.
23. Ledoux DR, Henry PR, Ammerman CB, et al. Estimation of the relative bioavailability of inorganic copper sources for chicks using tissue uptake of copper. J Anim Sci 1991;69:215-22.
24. Zulauf-Fischer I, Liesegang A, Haessig M, et al. Differences in bone mineral content and density between male and female budgerigars (Melopsittacus undulatus) during the non-reproductive season. J Vet Med A Physiol Pathol Clin Med 2006;53: 456-7.
25. Onyango EM, Hester PY, Stroshine R, et al. Bone densitometry as an indicator of percentage tibia ash in broiler chicks fed varying dietary calcium and phosphorus levels. Poult Sci 2003;82:1787-91.
26. Schreiweis MA, Orban JI, Ledur MC, et al. The use of densitometry to detect differences in bone mineral density and content of live White Leghorns fed varying levels of dietary calcium. Poult Sci 2003;82:1292-301.
27. Martinez-Cummer MA, Heck R, Leeson S. Use of axial X-ray microcomputed tomography to assess three-dimensional trabecular microarchitecture and bone mineral density in single comb white leghorn hens. Poult Sci 2006;85:706-11.
28. Zhang B, Coon CN. The relationship of various tibia bone measurements in hens. Poult Sci 1997;76:1698-701.
29. Zotti A, Rizzi C, Chiericato G, et al. Accuracy and precision of dual-energy x-ray absorptiometry for ex vivo determination of mineral content in turkey poult bones. Vet Radiol Ultrasound 2003;44:49-52.
30. Fleming RH, Korver D, McCormack HA, et al. Assessing bone mineral density in vivo: digitized fluoroscopy and ultrasound. Poult Sci 2004;83:207-14.
31. Korver DR, Saunders-Blades JL, Nadeau KL. Assessing bone mineral density in vivo: quantitative computed tomography. Poult Sci 2004;83:222-9.
32. Schreiweis MA, Orban JI, Ledur MC, et al. Validation of dual-energy X-ray absorptiometry in live White Leghorns. Poult Sci 2005;84:91-9.
33. Hester PY, Schreiweis MA, Orban JI, et al. Assessing bone mineral density in vivo: dual energy X-ray absorptiometry. Poult Sci 2004;83:215-21.
34. Johnston CC Jr., Slemenda CW, Melton LJ 3rd. Clinical use of bone densitometry. N Engl J Med 1991;324:1105-9.
35. Shahnazari M, Sharkey NA, Fosmire GJ, et al. Effects of strontium on bone strength, density, volume, and microarchitecture in laying hens. J Bone Miner Res 2006;21: 1696-703.

36. Forsman D. The raptors of Europe and the Middle East. A handbook of field identification. Princeton: University Press; 1998.
37. Glutz von Blotzheim U, Bauer K. Handbuch der Vögel Mitteleuropas. Band 4: Falconiformes. Wiesbaden: Akademische Verlagsgesellschaft; 1980.
38. Bettin H, Spieweck F. Die Dichte des Wassers als Funktion der Temperatur nach Einführung der Internationalen Temperaturskala von 1990. PTB-Mitteilungen 1990;3/90:195-6.
39. Naumann C, Bassler R. Methodenbuch Band III, Die chemische Untersuchung von Futtermitteln. Darmstadt: VDLUFA; 1976.
40. Nickel R, Schummer A, Seiferle E. Lehrbuch der Anatomie der Haustiere. Band V: Anatomie der Vögel. 2. Aufl. Berlin: Paul Parey; 1992.
41. Kirchgeßner M. Tierernährung. 10. Aufl. München: DLG; 1997.
42. Cubo J, Casinos A. Mechanical properties and chemical composition of avian long bones. Eur J Morphol 2000;38:112-21.
43. Krampitz G, Enbergs H, Petersen BK. Knochen- und Knorpelaufbau, Kalkstoffwechsel. In: Handbuch der Geflügelphysiologie. Mehner A, Hartfiel W, eds. Basel: Karger: 1983. pp 163-217.
44. Doyle JJ. Toxic and essential elements in bone - a review. J Anim Sci 1979;49:482-97.
45. Wedekind KJ, Baker DH. Zinc bioavailability in feed-grade sources of zinc. J Anim Sci 1990;68:684-9.
46. Cook ME, Sunde ML, Stahl JL, et al. Zinc deficiency in pheasant chicks fed practical diets. Avian Dis 1984;28:1102-9.
47. Lappalainen R, Knuuttila M, Lammi S, et al. Zn and Cu content in human cancellous bone. Acta Orthop Scand 1982;53:51-5.
48. Herzog K. Anatomie und Flugbiologie der Vögel. Stuttgart: Gustav Fischer; 1968.