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Osteomorphometry of the bones of the thigh, crus and foot in the New Zealand white rabbit (*Oryctolagus cuniculus*)

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Summary

The morphology and morphometry of the bones of the thigh, crus and foot of ten adult New Zealand white rabbits were investigated to outline the peculiarities of the species and attempt to establish a morpho-functional paradigm. The femur, tibia-fibular, tarsus, metatarsals and digits of the right and left limbs were extracted and properly macerated. Gross observations of the femur revealed the presence of three trochanters. The greater trochanter was considerably higher than the head of the femur. The femoral head possessed a *fovea capitis*. The distal part of the femur had prominent *condylus lateralis, condylus medialis, epicondylus lateralis* and *epicondylus medialis*. The femur had an average length of 8.230 cm (SD ± 0.086). The proximal aspect of the tibia possessed a prominent tuberosity and two condyles. Distal fusion of the tibia and fibula was observed since about half of the total tibia length. The distal articular surface of the tibia was about twice as wide medio-laterally than dorso-ventrally and deeply excavated to accommodate the relief of the talus trochlea. The right and left tibia length measured 9.100 cm (± 0.119) and 9.080 cm (± 0.120), respectively. Six distinct tarsal bones were observed. The foot was complete with four digits. The relative lengths of the metatarsal bones were III > II > IV > I. Various other parameters were measured. Bilateral asymmetry was observed in all the considered parameters.

Key words

Rabbit; mophometry; maceration; femur; tibia; fibular; foot.

Introduction

Rabbits belong to the family Leporidae of the order Lagomorpha. They are rather uniform in body proportions and stance. Rabbits tend to range in size from 25-50 cm in length and from 400-3,000 g in weight (Angerbjörn, 2004). There are over 100 breeds of rabbits, which are descendants of the European wild rabbit (*Oryctolagus cuniculus*) of which the majority used in biomedical research are of the New Zealand White breed (Wilber, 1999).

The skeleton of the rabbit is relatively fragile representing about 8 % of the body weight (Brewer, 2006). There have been various investigations of the macro-anatomy

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of the pectorial girdle of the rabbit (Özkan *et al.*, 1997), the whole skeletal system of some other mammals including the guinea pig and the rat (Özkan *et al.*, 1997), the mink (Dursun and Tipirdamaz, 1989), the badger (Hidaka *et al.*, 1998; Dinç, 2001) the porcupine (Yilmaz *et al.*, 1998), the hedgehog (Özkan, 2005), and the mole rat (Özkan, 2007). However, literature on the detailed macro-anatomical investigations of the hind limbs of the rabbit is meager.

The aim of this study was to investigate the morphology and morphometry of the long bones of the pelvic limb of the New Zealand White rabbit in detail. This may be an added contribution to knowledge in the area of osteomorphometrics of the Leporidae family, and offer a foundation for establishing a morpho-functional paradigm to understand the peculiar adaptation features of the species.

Materials and methods

The long bones of the hind limbs were obtained from ten New Zealand White rabbits. They were obtained from adult rabbits in the abattoir at the time of slaughter. The live weights of the animals were taken using a weighing balance (P1210 Mettler Instruments AG, Zurich, Swizerland) with a sensitivity of 0.1 g. After slaughter, the hind limbs were carefully dissected and the soft tissues attached to the long bones, such as muscles, fascia, tendons and ligaments, were removed with the use of a scalpel blade. Maceration of the bones was carried out by the method described by Salami *et al.* (2011) and recapitulated below.

The specimens were properly labelled and immersed in a macerating bucket filled with tap water. This was allowed to stand for 3 days after which the water was changed. To the changed water, 1-1.5 Molar Potassium hydroxide (KOH) was dissolved to facilitate total removal of the remaining soft tissues from the bones. The macerated bones were dried for 2-3 days after which they were boiled with detergent to remove bone marrow and fats. The bones were allowed to dry for the second time, bleached with Hydrogen peroxide (H_2O_2) and washed with a common commercial abrasive powder and brush. They were dried for 2-3 days.

Completely macerated bones were weighed using a top loading balance (Gallenkomp FA2104A, Leicestershire, U.K.) and photographs were taken using a digital camera (Canon PowerShot A630, 8.0 MP, PC1201, Canon, Tokyo, Japan). Morphometric descriptions of the femur and tibia-fibula bones were undertaken and recorded using Vernier calliper (sensitivity: 0.01mm, MG6001DC, General Tools and Instruments Company, New York, U.S.A.) and a metric ruler. All recorded weights, lengths and diameters were expressed as mean ± SD (standard deviation) and terminology was in accordance to *Nomina Anatomica Veterinaria* (2005).

Results

Femur

The femur was thin (Fig. 1). It had a small head (*caput ossis femoris*) which was ovoid to sub-spherical and supported by a distinct femoral neck. There was a small

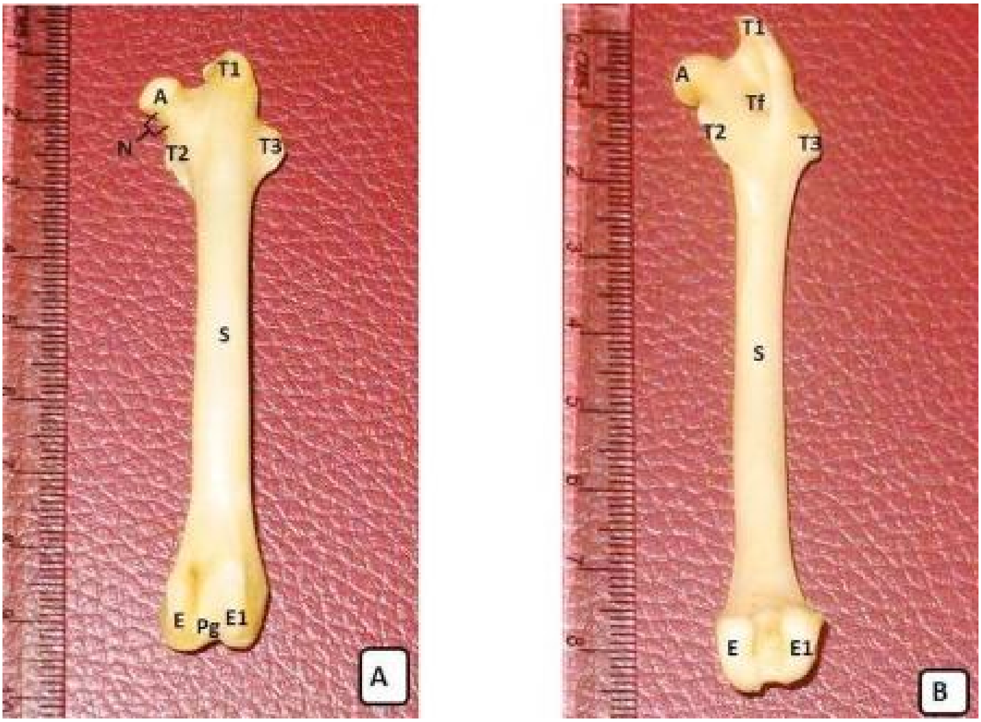

Figure 1 – A) Cranial surface of the femur, B) Caudal surface of the femur: A - Head of femur, N - Neck of femur, T1 - Greater trochanter, T2 - Lesser trochanter, T3 - Trochanter tertius, Tf - trochanteric fossa, S – Femoral shaft, E & E1 - medial and lateral condyles, Patella groove.

fovea (*fovea capitis*) on the femoral head. The articular surface of the femur did not extend into the neck of the femur. There were three trochanters. The greater trochanter (*trochanter major*), the lesser trochanter (*trochanter minor*) and a third trochanter (*trochanter tertius*) which formed a solid crest running from the greater trochanter to the femoral shaft. Laterally, the greater trochanter projected distinctly above the head of the femur and also extended caudo-medially. An intertrochanteric ridge (*crista intertrochanterica*) was present between the lesser and greater trochanter. The proximal extent of the third trochanter was at the level of the distal termination of the greater trochanter. Distally, the femur was narrower. The patella groove was deep, narrow, long and extended a little proximally along the shaft of the femur. The medial ridge was observed to be higher than the lateral ridge. *Condylus lateralis, condylus medialis, epicondylus lateralis, epicondylus medialis, linea* and *fossa intercondylaris* were prominent. Caudally, a little proximal to the condyles were the medial and lateral supracondyloid tuberosities. Also caudal to the condyles were two sesamoid bones (*sesamoid bones of Vesal*) embedded in the tendons of origin of the gastrocnemius muscle. Various parameters including the length of the femur, width of proximal femur, width of distal femur, width of femoral shaft and width of femoral head were measured and recorded as seen in Table 2.

Table 1 – Weight of live body, femur and tibia of New Zealand white rabbit.

	1	2	3	4	5	6	7	8	9	10	Mean (± SD)
AWT (g)	1750.00	1750.00	1510.00	1700.00	1650.00	1550.00	1650.00	1950.00	1500.00	2250.00	1726.00 (± 227.75)
WRF (g)	4. 252	3.758	3.377	4.100	3.416	3.721	3.821	4. 233	3.310	4.327	3.832 (± 0.384)
WLF (g)	4.378	3.852	3.403	4.140	3.602	3.708	3.756	4. 275	3. 274	4. 256	3.864 (± 0.384)
WRT/F (g)	3.814	3.313	3.029	3.816	2.995	3.510	3.374	3.981	3.088	3.901	3.482 (± 0.378)
WLT/F (g)	3.984	3.568	3.037	3.808	2.940	3.400	3.335	3.965	3.094	3.839	3.497 (± 0.394)

AWT - Live animal weight, WRF - Weight of right femur, WLF - Weight of left femur, WRT/F - Weight of right tibia and fibula, WLT/F - Weight of left tibia and fibula.

Tibia-fibula

The tibia was relatively longer than the femur. The articular surface of the proximal aspect of the tibia (*facies articularis proximalis*) was roughly heart-shaped in outline when viewed proximally. The tibia condyles (*condylus medialis* and *condylus lateralis*) appeared approximately equal in surface area. However, the lateral condyles were slightly elevated above the medial condyles. The tibia tuberosity (*tuberositas tibiae*) to which the patella ligament attaches was narrow and projected ventrally. The tibia and fibula were separated by a wide interosseous space (Fig. 2). The fibula was a very slender bone with a facet on the proximal tibia situated just dorso-caudal to the lateral tibia condyle. The fibula was independent in its proximal part and fused with the tibia at about half length of the latter bone. Distal to the point of tibia-fibula fusion, the tibia shaft appeared narrow. The distal articular surface of the tibia was about twice as wide medio-laterally as dorso-ventrally and deeply excavated to accommodate the relief on the talus trochlea. The deep grooves were separated by the medial and lateral malleoli. Various parameters were measured including tibia length, width of proximal tibia, width of distal tibia, tibia shaft diameter, and length of fibula. In all the measured parameters bilateral asymmetry was observed, however, the differences appeared to be very slight (Table 2).

Ossa tarsi

There were six distinct tarsal bones (Fig. 3). The proximal row consisted of the tibia tarsal (*talus*) and the fibula tarsal (*calcaneus*) bones. The talus was irregular in form. The calcaneus appeared as the largest bone of the tarsus. It was elongated and formed the point of the hock. It had two major parts, a proximal part that formed the calcaneal tuber (point of the hock), and a distal extremity which bore a facet for

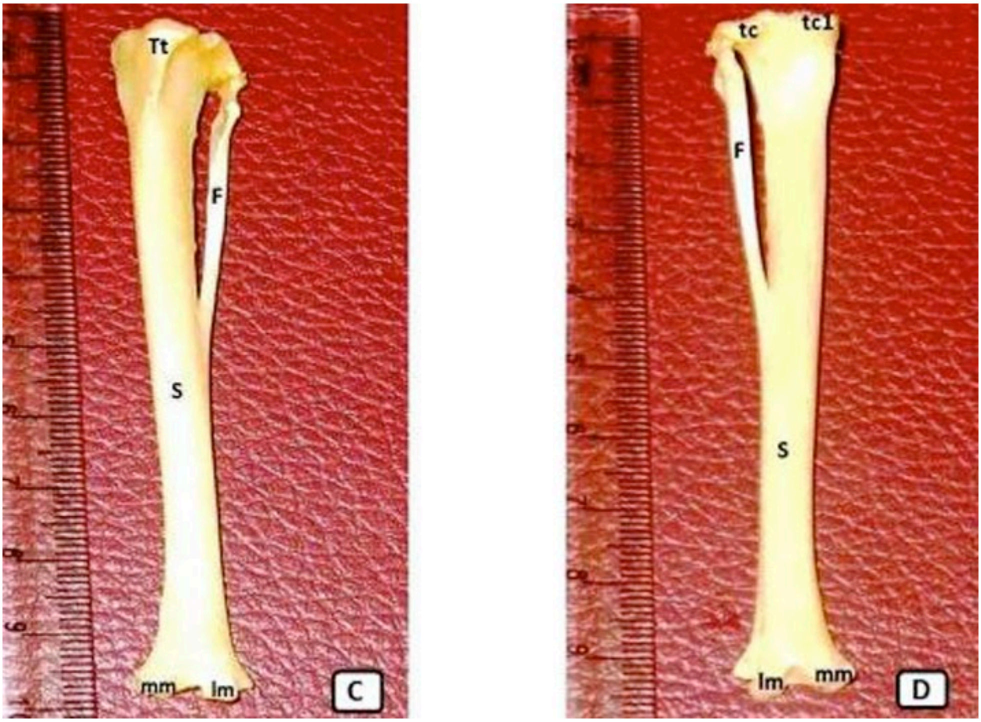

Figure 2 – A) Cranial surface of the tibia, B) Caudal surface of the tibia: Tt - Tibia tuberosity, F - Fibula, S - Shaft of tibia, mm - Medial malleolus, lm - lateral malleolus, tc - lateral tibia condyle and tc1 - medial tibia condyle.

Table 2 – Mean values of various femur and tibia parameters measured.

Parameters (cm)	RIGHT (± SD)	LEFT (± SD)
LF	8.230 (± 0.271)	8.230 (± 0.263)
WPF	1.539 (± 0.131)	1.583 (± 0.111)
WDF	1.329 (± 0.041)	1.303 (± 0.036)
WFS	0.629 (± 0.399)	0.629 (± 0.051)
WFH	0.663 (± 0.077)	0.643 (± 0.034)
TL	9.100 (± 0.377)	9.080 (± 0.379)
WPT	0.911 (± 0.057)	0.926 (± 0.066)
WDT	1.250 (± 0.130)	1.174 (± 0.063)
TSD	0.590 (± 0.219)	0.579 (± 0.224)
FL	3.995 (± 0.177)	4.089 (± 0.397)

LF - Length of femur, WPF - width of proximal femur, WDF - width of distal femur, WFS - Width of femoral shaft, WFH - width of femoral head, TL - tibia length, WPT - width of proximal tibia, WDT - width of distal tibia, TSD - tibia a shaft diameter, FL - length of fibula.

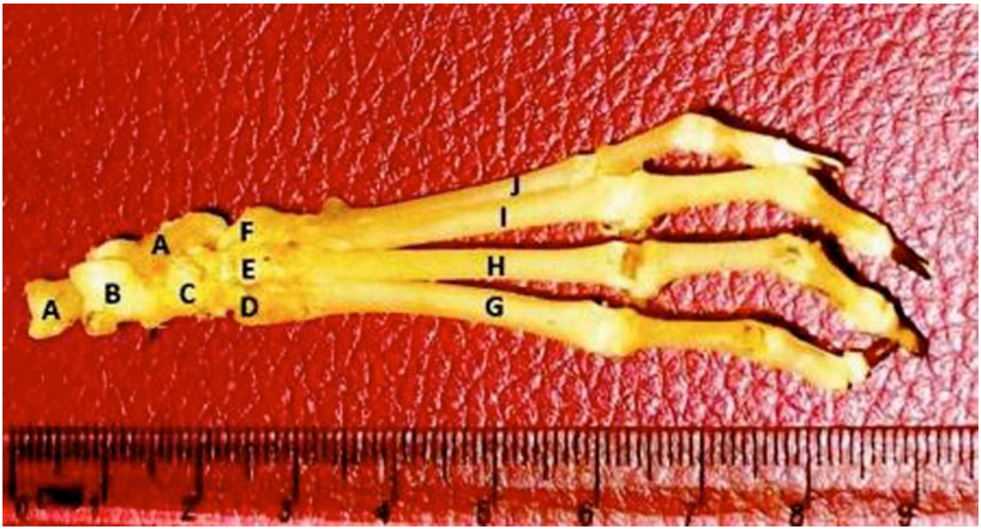

Figure 3 – A) Calcaneous, B) talus, C) Distal central tarsal bone, D) *Os tarsale I*, E) *Os tarsale II*, F) *Os tarsale III* (fused third and fourth tarsals), G) metatarsal I, H) metatarsal II, I) metatarsal III, J) metatarsal IV.

articulation with the fused third and fourth tarsals. The dorsal border bore a coracoid process which presented facets for articulation with the talus.

There was a central tarsal bone distal to the talus. The distal row of bones from medial to lateral consisted of an *os tarsale* I, *os tarsale* II and a fused *os tarsale* III and IV.

Ossa metatarsi

The foot was complete with four digits and there were four distinct metatarsal bones lying between the tarsal bones and the phalanges. The relative lengths of the tarsal bones were III > II > IV > I. There were two plantar located sesamoid bones at each metatarsophalangeal joint (Fig. 3).

Ossa digitorum pedis

All four digits had three phalanges each. The distal phalanges were arched and pointed to accommodate the curved nails.

Discussion

The greater trochanter was distinctly higher than the head of the femur in all the animals studied. Pentrot *et al.* (2008) reported that the high greater trochanter provides a long lever arm for the deep gluteals. It has also been reported that the height of the greater trochanter would also limit the potential range of thigh abduction (Taylor, 1976; Rose, 1999; Argot, 2002; 2003; 2004). The lesser trochanter projected dorso-medially, similar to the common configuration observed in specialized runners and jumpers (Taylor, 1976; Rose, 1999; Argot, 2002). This suggests an emphasis on fore and backward flexion of the hip (Pentrot *et al.,* 2008). The very well developed third trochanter located on the proximal one-third of the length of the femur was similar to the reports of Özkan (2002a) in the study of the hind limb of the mole rat. This also appeared similar to the observations of Olude *et al.* (2009) on the hind limb of the African giant rat. The morphology of the trochanters was similar to the observations of Olude *et al.* (2009). This morphology is consistent with the ability for rapid flexion and extension of the hip through the increase of lever arm lengths. However, the femoral head possessed a fovea capitis which is quite different from the report of the femoral head of the African giant rat (Olude *et al.,* 2009) and the porcupines (Yilmaz *et al.*, 1999). According to the result of this study, the mean absolute length of the femur was 8.230 cm while that of the tibia was 9.09 (Table 2). The relatively short femur compared to the tibia fibula in absolute terms was consistent with that of runners. This is in line with the pattern of lengthening distal limb elements to increase stride (Taylor *et al.*, 1974; Hildebrand, 1985; Argot, 2004; Berman, 1985).

The fibula of the New Zealand white rabbit is a slender bone separated from the tibia and fused with the tibia for about its distal half. It has been reported that in some species, for instance the Erinaceus the tibia and fibula are fused at the distal end (Saunders and Manton, 1969), and in porcupines the fibula is fused with the tibia at the proximal portion (Yilmaz *et al.*, 1999). Pentrot *et al.* (1999) reported that the distal fusion of the tibia and fibula is an adaptation to stress on the distal crus. This has been documented as an adaptational feature characteristic of diggers as well as leapers (Barnett and Napier, 1953; Argot, 2002). The large deeply excavated attachment site for the tibialis anterior muscle on the lateral side of the proximal tibia suggests powerful dorsiflexion of the ankle, a feature of the runner taxa as well as fossorials and semi-aquatic taxa (Pentrot *et al.*, 2008).

It has been documented that seven irregular bones varying greatly in size shaped the tarsus in the hedgehogs (Özkan, 2002b) and squirrels (Özdemir and Atalar, 2003). Eight tarsal bones exist in the porcupines (Yilmaz *et al.*, 1999), mole-rats (**Özkan 2002a**) and chinchillas (Cevik-Demirkan *et al.*, 2007). However, we observed 6 tarsal bones in our study. The foot consisted of four fully developed digits in contrast to five digits in the porcupines (Yilmaz *et al.*, 1999), martens (Atalar and Özdemir, 2002), hedgehogs (Özkan, 2002b) and squirrels (Özdemir and Atalar, 2003).

The relative lengths of the metatarsal bones were IV > III > II > V > I in the hedgehogs (Özkan, 2002b) and the African giant rat (Olude *et al.*, 2009), and III > IV > II > V in the chinchilla (Cevik-Demirkan *et al.*, 2007). This was quite different from our study (III > II > IV > I, in the rabbit).

There existed one sesamoid bone (*sesamoid bones of vesal*) on the *condylus lateralis* and one on the *condylus medialis* of the distal femur. This is similar to the report of Atalar and Özdemir (2002) in martens**.** Two plantar sesamoid bones were also located at each metatarsophalangeal joint. This is similar to the report in rats (Özkan, 2002a) and porcupines (Yilmaz *et al.*, 1999).

Our study has attempted to buttress the importance of the hind limb of the rabbit in the light of its digging, leaping and running activities. The developed features observed in the long bones of the hind limb of the rabbit explain its ability for quick movement in the wild as an adaptation for keeping away from potential predators. In the laboratory, the rabbit has been tagged as an extremely valuable model for study hence there is an indication for an exhaustive exploration of its anatomy. It is therefore hoped that the data generated here may contribute to the current knowledge in the area.

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