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Volume calculation of the cattle (*Bos taurus L.*) and the water buffalo (*Bos bubalis L.*) metapodia with stereologic method

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*In this study, stereological volume estimations using 26 cattle metapodia (26 metacarpal and 26 metatarsal bones) and 8 water buffalo metapodia (8 metacarpal and 8 metatarsal bones) were made. For this purpose metapodia were parallel sectioned at 1 cm intervals according to Cavalieri principle. Grids with 0.4 cm probe intervals were superimposed on top of these sections and the matching points were counted. All of the bone structures and medullar cavity volumes were calculated with the data obtained from a formulation ($V = t \times a(p) \times \sum P$) as a spreadsheet using Microsoft Excel® Windows XP. In addition, percent ratio of this volume to whole bone volume was calculated. The mean ratio of bone marrow space to whole bone structure volume equals 15% in all of the cattle and buffalos. The difference between whole bone volumes of cattle and water buffalo was significant ($p < 0.05$) while the difference in volume of medullary cavity (*cavum medullare*) was not significantly different between the two investigated species. The aim of current study is to present a new method that can be used for the volumes calculation of whole bones and medullary cavity in metapodial bones and their percentages.* (Folia Morphol 2015; 74, 3: 335–339)

Key words: metapodium, stereology, bone marrow

INTRODUCTION

Beside the fact that, animal fats are not currently used widely in human diets, they had considerable importance in daily consumption of ancient people since their diets were based principally on animal origin food. The skeleton itself, is an important fat resource, especially the fats in the bone marrow of the medullary cavity and the spongy bones. Metacarpal and metatarsal bones were important due to its role as a fat source for the past societies, in which the animal husbandry was the dominant factor in community economy, especially before Secondary Products Revolution [9, 13, 24]. Since the fat provides more calories compared to proteins and carbohydrates [7, 12], fats sources of any kind were highly important in the past, especially for people who had strongly limited nutrition sources [19, 20]. The fats are preferred in diet, because of the facts that they are easily metabolised and its small quantity sufficiency for energy supply [19]. It is known, that the concentration of fatty acids present in the bone marrow exhibits variations [14]. Oleic acid, which is one of the

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unsaturated fatty acids are found more in bones from the distal parts of appendices such as metapodia [13, 24]. Oleic acid has low melting point, and compared to the other fatty acids, its nutritional value and flavour is higher [3]. The fattened animals are known to have more bone marrow compared to animals in poor condition [27]. Nevertheless, knowing the volume of metapodial medullary cavity in an animal, even in an emaciated one, is important to estimate the amount of bone marrow [18]. In the classical methods where bone marrows has been attempted to be removed using bone wall perforations, some difficulties are faced due to trabecular bone. Similarly, in the efforts to fill the cavity with water to estimate the volume, troublesome results were obtained due to spread of the water to spongy parts of the bone [18]. With the exception of classical methods where the bone marrow is evacuated [8, 18], stereological methods are preferred over other planimetric methods because it provides opportunity to evaluate many other aspects of the bones and yields more realistic estimations. Stereological methods have been used in histological and morphometric studies [21, 26].

The objective of this study was to present a new method that can be used for calculation of volumes of whole bones and medullary cavity in metapodial bones and their percentage participations.

Figure 1. Cross-sections of metapodia.

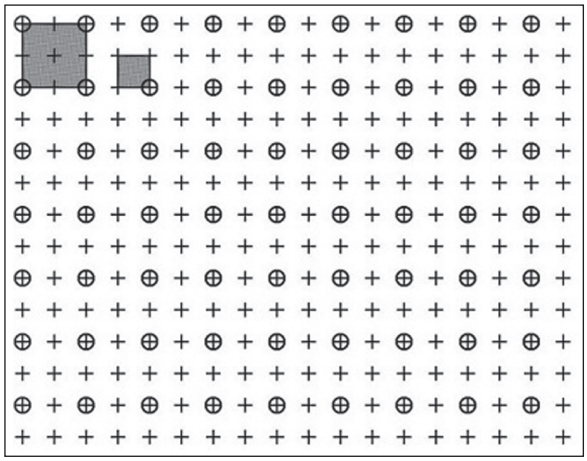

Figure 2. A combined point counting grid with 1/4 area fraction. While an encircled cross represents a large area, each cross without a circle represents 1/4 fraction of the large areas.

MATERIALS AND METHODS

In this study, the material consists of the left metapodia coming from 26 cattle (26 metacarpal and 26 metatarsal bones) and 8 buffalos (8 metacarpal and 8 metatarsal bones) collected from different slaughterhouses located in Istanbul. All of the buffalos and cattle were newly completed their development to reach adulthood. The dual-purpose (milk-meat) Holstein-Friesian cattle in Turkey were used in this study. According to Cavalieri principle, the metapodia were sectioned in parallel at 1 cm intervals along the bone long axis for stereological calculation of the volume (Fig. 1). For the medullary cavity, grids with 0.2 cm intervals, and for the whole bone, grids with 0.4 cm intervals were used (Fig. 2).

The cross sections were lined up for placing point counting great on their top (Fig. 3). The grids were randomly thrown on top of the cross sections and the points on the cross sections were counted. Using these data, whole bone volumes and medullary cavities volumes were calculated as a spreadsheet using Microsoft Excel® program. Mean value, standard deviation, and mean of medullary cavity were computed for cattle and water buffalo metapodia.

Coefficient error (CE), which is used for decision on the concentration of the points on the grid, was calculated. Using these calculations per cent ratio of the medullary cavity with regards to whole bone volume was calculated for the metacarpal and metatarsal bones.

The following formula was used to estimate the volumes of total bone and its medullary cavity by counting the intersecting points between the grids and the regions of interest [11]:

$$V = t \times a(p) \times \sum P$$

where: t — section thickness (1 cm); a(p) — the area represented by a point in the grid (0.16 or 0.04 cm^2); $\sum P$ — total number of points hitting the surface area of section.

In statistical data estimation (mean value, standard deviation, coefficient of variation), SPSS 8.0 was used.

Figure 3. The cross-section of the bones lined up for superimposition of the grid membrane.

RESULTS

After calculations, volumes of the bones in cattle were stated as 43.08 ± 8.701 cm^3 for whole metacarpus, and 6.35 ± 2.230 cm^3 for medullary cavity of metacarpus, 52.02 ± 10.998 cm^3 for whole metatarsus, and 7.44 ± 2.185 cm^3 for medullary cavity of metatarsus. The results for water buffalo were 36.95 ± 6.421 cm^3 for whole metacarpus, and 5.45 ± 1.770 cm^3 for medullary cavity of metacarpus, 44.29 ± 7.817 cm^3 for whole metatarsus, and 6.98 ± 1.946 cm^3 for medullary cavity of metatarsus. Average per cent ratio of the medullary cavity volume to whole bone volume was 15% in all metapodia for both species. A significant difference between the whole bone volumes of cattle and water buffalo ($p < 0.05$) was proved. However it should be noticed, that the medullary cavity volumes did not significantly differ between the two species (Table 1).

CE values that were effective in deciding the point frequency on the grids were found as 2% for whole metacarpus, as 5% for medullary cavity of the metacarpus in cattle. The corresponding values for metatarsus were 1% and 5%. In water buffalo, CE equals 2% for whole bone and 5% for the medullary cavity in the metacarpal bones.

DISCUSSION

In the case of carbohydrate deficiency, lipids became the major source of energy [19]. It's well known,

Table 1. The comparison of volumes of whole bone and cavum medullare in metacarpus and metatarsus of cattle and water buffalo

	Metacarpus			Metatarsus		
	TBV	CMV	%	TBV	CMV	%
Cattle (Bos taurus L.), n = 26						
Mean	43.08[a]	6.35[a]	0.15	52.02[a]	7.44[a]	0.14
Standard deviation	8.701	2.230	0.034	10.998	2.185	0.026
Minimum	18.80	2.50	0.10	25.52	2.84	0.09
Maximum	57.92	10.14	0.22	71.44	10.54	0.19
Coefficient of variation	20.20	35.12	22.66	21.14	29.36	18.46
Coefficient error	0.02	0.05	–	0.01	0.04	–
Water buffalo (Bos bubalis L.), n = 8						
Mean	36.95[b]	5.45[a]	0.15	44.29[b]	6.98[a]	0.16
Standard deviation	6.421	1.770	0.028	7.817	1.946	0.021
Minimum	29.12	3.72	0.13	33.36	4.76	0.14
Maximum	46.88	9.24	0.21	57.20	10.16	0.20
Coefficient of variation	17.38	32.46	19.15	17.65	27.89	13.36
Coefficient error	0.02	0.05	–	0.02	0.05	–

TBV — total bone volume; CMV — cavum medullare volume; [a, b] The difference between the mean values of measurements is statistically significant ($p < 0.05$)

when nutrition with foods of animal origin is practised, the most important lipid source is the bone marrow lipids [20]. The main sources of these lipids are the bone marrow and the spongy bone [20]. The lipids of the distal extremities such as metapodia are often preferred for nutrition because they are highly nutritious, rich in unsaturated fatty acids (particularly oleic acid), and show variations in types and concentrations of the fatty acids [12, 14]. This preference was not only today's, but especially that of past societies [16]. Probably because of this preference, it has been reported that volumetric calculations were made by extraction method to predict the amount of bone marrow in distal extremities [13, 14]. However, stereological methods are considered as a more acceptable scientific approach for these calculations [15, 18], because the classical methods revealed some difficulties such as injecting fluid to the cavity of bone. By using Cavalieri principle, which is a stereological method, not only the volume of bone marrow but also volume of the bone can be calculated. For this method, a scientifically acceptable CE value (should be < 5%) is required [19, 25, 26]. Using appropriate point concentration ruler that is used to obtain the CE value, sufficient numbers of cross-sections can be sampled. In our study, CE value of < 5% was achieved by using this method for both cattle and water buffalo metapodia. These values indicate that the numbers of cross sections samples were satisfactory.

The results of the current study indicated that there was a significant ($p < 0.05$) difference between the total volumes of metapodia and metatarsi of water buffalo and cattle. Possible reason of higher total bone volume results in cattle can be caused with higher quantity of the compact bone in cattle as well as larger size of cattle breeds that were used in the study. This indicates the presence of a positive correlation between the size of the body and the metapodia. Despite the difference in total bone volume, there was no significant difference between the volumes of metapodial medullary cavity of the two species. Although a relatively greater value was observed in cattle, this difference should not be recognised as a discriminative feature between two species. This difference which was not found as significant was probably the result of the body sizes variability among investigated animals. Another interesting finding in this study is that the medullary cavity volume compared with the total bone volume ratio was around 15% in both of the metacarpus and metatarsus of the both species. Although total bone volume and the medullary cavity volume were higher ($p < 0.05$) in cattle than those values computed for water buffalo, our findings indicate that the ratio within the species remains constant, and this should be seen as a situation related to the body size. Although presence of a negative correlation between the width of medullary cavity and age in cattle has been known [22], effects of the individual body have not been taken into consideration in these relations. The cattle and water buffalos used in our study were the adult animals that just completed their development and growth. Although their body size and genders varied, the ratio of medullary cavity: total bone volume calculated in these animals were almost the same. It has been thought that the quantity of bone marrow that will be obtained from these bones would be expected to be the same. Considering the effect of gender on the metapodia, morphometric differences between bulls and cows have been known [1]. Further studies have been warranted to elucidate the gender differences role in the bone volumes. This study provides an opportunity to evaluate bone volumes in both water buffalos and cattle, regardless of the gender. It is noteworthy to underline that, as expected, there were differences in the bone and the medullary cavity volumes between the individual animals, but not significant. In studies about metapodia, the dimensional index calculations were made usually by using morphometric measurements. For this purpose, both radiographic and direct osteometric methods have been used [4–6, 10, 17, 23, 28]. In these studies, morphological differences in metapodia [22] together with the asymmetry of bones were evaluated and presence of a clear asymmetry in the metacarpus has been reported [2]. However, volume calculations in these bones by stereological methods have remained limited with classical methods (fluid replacement principle; the amount of fluid injected into the medullary cavity or the amount of overflowed fluid). Regarding the morphometric measurements, the reason why the correlation between the size of medullary cavity and the bone width was different in both metacarpus and metatarsus has been explained by biomechanical structures of these bones [22]. Change of the location of compact bone in both bones is believed to play a role in this correlation. However, the asymmetry observed during the morphometric measurements during the volume calculations still remains as a question mark. It has not been focused on whether there was a ho-

motypic variation between the volume measurements. However, different species have been compared.

CONCLUSIONS

Evaluation of volumetric differences in future studies that will be planned on asymmetry or homotypical variation studies will be a valuable contribution to this scientific divagations area. The calculations of total bone volume and the medullary cavity volume in this study, will not only enable estimating the amount of bone marrow, but also contribute to the biomechanical studies of animals. The relation between the compact bone thickness and the medullary cavity volume may be of a valuable importance in biomechanical some approaches.

REFERENCES

1. Bartosiewicz L (1984) Sexual dimorphism of long bone growth in cattle. Acta Vet Hung, 32: 135–146.
2. Bartosiewicz L, Van Neer W, Lantacker A (1993) Metapodial asymmetry in Draft Cattle. Int J Osteoarchaeol, 3: 69–75.
3. Binford LR (1978) Nunamiut ethnoarchaeology. Academic Press, New York.
4. Davies HMS, Watson, KM (2005) Third metacarpal bone laterality asymmetry and midshaft dimensions in Thoroughbred racehorses. Aust Vet J, 83: 224–226.
5. Davis SJM (1996) Measurements a group of adult female Shetland sheep skeletons from a single flock: a baseline for zooarchaeologists. J Archaeol Sci, 23: 593–612.
6. Davis SJM (2000) The effect of castration and age on the development of the Shetland sheep skeleton and a metric comparison between bones of males, females and castrates. J Archaeol Sci, 27: 373–390.
7. Erasmus U (1986) Fats and oils: the complete guide to fats and oils in health and nutrition. Alive Books, Vancouver, Canada.
8. Gong JK, Ries W (1970) Volumetric composition of the rabbit skeleton. Anat Rec, 167: 79–86.
9. Greenfield HJ (2010) The secondary products revolution: the past, the present and the future. World Archaeology, 42: 29–54
10. Guintard C (1998) Ostéométrie des métapodes de bovins. Rev Méd Vét, 149: 751–770.
11. Gundersen HJ, Jensen EB, Kieu K, Nielsen J (1999) The efficiency of systematic sampling in stereology-reconsidered. J Microsc, 193: 199–211.
12. Mead JF, Alfin-Slater RB, Howton DR, Popjak G (1986) Lipids: chemistry, biochemistry and nutrition. Plenum Press, New York.
13. Meng MS, West GC, Irving L (1969) Fatty acid composition of caribou bone marrow. Comp. Biochem Physiol, 30: 187–191.
14. Munro ND, Bar-Oz G (2005) Gazelle bone fat processing in the Levantine Epipalaeolithic. J Archaeol Sci, 32: 223–239.
15. Nieminen M, Laitinen M (1986) Bone marrow and kidney fat as indicators of condition in reindeer. Rangifer, Special Issue, 7: 219–226.
16. Onar V, Belli O (2007) Animal Fauna of the Van Region in the First – Half of the First Millenium B.C. According Castle", II. Van Gölü Havzası Sempozyumu, 04–07 Eylül 2006 Bitlis, (ed. O. Belli), Bitlis Valiliği İl Kültür ve Turizm Müdürlüğü Yayını, Nr. 17, Desen Ofset Matbaası, Ankara, 68–77.
17. Onar V, Pazvant G, Belli O (2008) Osteometric examination of metapodial bones in sheep (Ovis aries L.) and goat (Capra hircus L.) unearthed from the Upper Anzaf Castle in Eastern Anatolia. Rev Méd Vét 159: 150–158.
18. Outram A, Rowley-Conwy P (1998) Meat and marrow utility indices for horse (Equus). J Archaeol Sci, 25: 839–849.
19. Outram AK (1999) A comparison of Paleo-Eskimo and Medieval Norse bone fat exploitation in Western Greenland, Arctic Anthropol, 36: 103–117.
20. Outram AK (2002) Bone fracture and within-bone nutrients: an experimentally based method for investigating levels of marrow extraction. In: Miracle P, Milner N eds. Consuming passions and patterns of consumption. McDonald Institute for Archaeological Research, Cambridge, pp. 51–64.
21. Ömer H, Alpak H (2012) Cranial volume estimation using stereological method in Kivircik sheep and its correlations between skull parameters. J Fac Vet Med Istanbul Univ, 38: 107–119.
22. Páral V, Tichý F, Fabiš M (2004) Functional structure of metapodial bones of cattle. Acta Vet Brno, 73: 413–420.
23. Peters J (1998) "Römische Tierhaltung und Tierzucht: eine Synthese aus archäozoologischer Untersuchung und schriftlich-bildlicher Überlieferung". Passauer Universitätsschriften zu Archäologie, Band 5. Verlag Marie Leidorf, Rahden, Westfalen.
24. Pond CM, Mattacks CA, Colby RH, Tyler NJC (1993) The anatomy, chemical composition and maximum glycolytic capacity of adipose tissue in wild Svalbard reindeer (Rangifer tarandus platyrhynchus) in winter. J Zool Lond, 229: 17–40.
25. Sahin B, Emirzeoglu M, Uzun A, Incesu L, Bek Y, Bilgic S, Kaplan S (2003) Unbiased estimation of the liver volume by the Cavalieri principle using magnetic resonance images. Eur J Radiol, 47: 164–170.
26. Thomsen JS, Laib A, Koller B, Prohaska S, Mosekilde L, Gowin W (2005) Stereological measures of trabecular bone structure: comparison of 3D micro computed tomography with 2D histological sections in human proximal tibial bone biopsies. J Microsc, 218: 171–179.
27. Verme LJ, Holland JC (1973) Reagent-dry assay of marrow fat in white-tailed deer. J Wildlife Manage, 37: 103–105.
28. Walter LJ, Davies HMS (2001) Analysis of a radiographic technique for measurement of equine metacarpal bone shape. Equine Vet J Suppl, 33: 141–144.