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Morphometry of the Cervical Spine of Emu Using the Hydrostatic Method

Morfometría de la Columna Cervical de Emu Usando el Método Hidrostático

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SUMMARY: The study aimed at estimating the values of basic metric traits of emu cervical vertebrae. The study was conducted on the vertebrae of 6 male and 10 female emus being fourteen years old. Osteometric measurements were performed with electronic callipers, while the hydrostatic method was used to assess the density and volume of each vertebra. The sex of birds was considered a source of variation. The cervical spine had 17 vertebrae. Dimorphism was found in basic metric traits between analogous emu vertebrae of both sexes. The female vertebrae were characterised by significantly ($P \leq 0.05$ and $P \leq 0.01$) greater length, breadth and height than the male ones. No dimorphic differences were found in the volume of bone mass for vertebrae 1 to 8, whereas female vertebrae 9 to 17 had greater ($P \leq 0.05$) volume compared to the male ones. Correlation coefficients for body weight, vertebra volume and spinal canal capacity were weak. The sum of the length of vertebral bodies determining the length of neck showed significantly ($P \leq 0.01$) longer necks in female emus. No narrowing and extensions of the vertebral canal for the spinal cord running in it was found throughout the whole cervical spine.

KEY WORDS: Cervical spine; Cervical vertebrae; Hydrostatic method; Ratiatae; Palaeognathae; Emu.

INTRODUCTION

In the birds in which the neck is a flexible long structure (Cobley *et al*., 2013), the number of vertebrae is highly variable. Basically, there can be from 9 cervical vertebrae – as in songbirds (Ferens & Wojtusiak, 1960) – to 25 ones – as in swans (Benoit *et al*. 1950; Woolfenden 1961). Cervical vertebrae in these animals are small bone units, which makes that the spinal canal is not an easy object to study, in morphological studies of their skeletons, while the methods of measurement are imprecise. It seems that this is a sufficient reason to justify the fact of scarce research in this area and as a result the scarcity of publications on this topic. Despite the many studies conducted on the skeletons of birds belonging to the superorder Ratiatae in the subclass Palaeognathae, such as ostrich or emu, and morphologically large ones, there is no reference to exact measurements of their vertebrae and spinal canal (Sales, 2006; Leite *et al*., 2012) or they are only insignificant and not very precise mentions (Cobley *et al.*; Kumar & Singh, 2014). In mammals, research of the vertebrae is limited to estimating the values of linear measurement in comparative morphometric studies on other species. If vertebrae are weighed, it is only to determine their own weight, not answering the question to which of the spine sections the most bone mass falls per unit length (Sasan *et al*., 2014). This question is interesting only because of the role of the cervical spine (Bogduk & Mercer, 2000), constituting a lever for the head of species in which it is, for instance, a tool of defense. The above arguments have given rise to analyzing the emu vertebrae and their bone mass volume and cervical spinal canal capacity. However, the primary goal was to obtain information being useful in studies on sexual dimorphism in the emu skeleton and to introduce the measurement methods into osteometric analyses that allow establishing the reference values for the assessment of maturation and its degree in birds.

MATERIAL AND METHOD

The study was conducted on cervical vertebrae of the spine of male (n=6) and female (n=10) emus being 14 years old, used for reproduction, from a breeding flock kept at the Department of Poultry and Ornamental Birds, Faculty

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of Biotechnology and Animal Husbandry, West Pomeranian University of Technology in Szczecin, Poland. The average body weight of male emus was 33.87 kg ± 4.67 (28.21 – 40.11kg), and that of female ones was 40.94 kg ± 5.35 (33.63 – 51.01 kg). Osteometric measurements were performed three times with electronic callipers, and the height, breadth and the greatest length of each vertebra was measured (their sum determined the length of cervical spine), as well as its cranial breadth and caudal breadth, and cranial articular surface breadth and caudal articular surface breadth. The callipers were also used to measure the length of the vertebral canal, while the surface area of the vertebral foramen was estimated using MultiScan software. The capacity of each vertebral canal was determined by multiplying the length of the vertebral canal by the surface area of the vertebral foramen. The hydrostatic method was used to estimate the density and volume of each vertebra. Measurements with the hydrostatic method were performed at 21 °C and the water density ρc = 0.99802 ($\frac{g}{cm^3}$). They consisted in drying the bone at 40 °C for 24 hours, weighing a dry sample in air to determine the mass m_0, soaking the samples in water for 24 hours, weighing a sample in liquid (water) to determine the mass m^2, and then weighing the water-saturated sample in air to determine the mass m_2. The calculations were made assuming that apparent density is defined as the ratio of the weight of dry sample to its total volume, including the measurements expressed in ($\frac{g}{cm^3}$), according to the following formula:

$$\rho_p = \frac{m_0}{(m_1 - m_2)\times\rho_c}$$

where:

ρ_p – apparent density of material ($\frac{g}{cm^3}$)
m_2 – weight of sample being weighed in water (g),
m_1 – weight of sample saturated with water being weighed in air (g),
m_0 – weight of dry sample being weighed in air (g),
ρ_c – density of liquid at the temperature of measurement.

In order to determine the bone volume, calculation was made according to the following formula: $V = \frac{m_0 - m_2}{\rho_c}$ (cm^3).

The measurements made allowed estimation of the values of the following indicators: mean vertebra bone mass volume, average vertebral canal capacity, and correlation between vertebral canal capacity and the length of vertebra. The sex of birds was assumed in the calculations to be a source of variation. In the absence of normal distribution and heterogeneity of the variance of metric traits, the significance of differences between groups was estimated using the Mann-Whitney non-parametric U test from Statistica software package (v.13.1PL).

RESULTS

The results obtained are presented in Table I to VIII. The cervical spine had 17 vertebrae. A particular morphologically distinct feature of the first vertebra was observed, consisting in the fact that the breadth and height of this vertebra exceeded almost three times its length, and the surface area of the vertebral foramen of this vertebra exceeded the value being estimated for the second vertebra (Table I and II) and was greater than the surface area of the fourth vertebra in the male emus, but comparable with that of the third vertebra in the female emus. The results presented in Table I indicate a significant ($P\leq0.05$ and $P\leq0.01$) dimorphism in basic metric traits between analogous vertebrae of the cervical spine of the emus of both sexes. The vertebrae of female emus were characterised by greater length, breadth and height compared to those of male emus. Table II presents the values of morphological traits for respective cervical vertebrae. The measurements of the cranial and caudal articular surface breadth and the surface area of vertebral foramen did not show any dimorphic differences. No dimorphic differences were found in the bone mass volume of cervical vertebrae 1 to 8 either, while the vertebrae 9 to 17 of female emus were characterized by significantly greater ($P\leq0.05$) bone mass volume compared to those of male emus (Table III). Female emus also significantly ($P\leq0.05$ and $P\leq0.01$) dominated over the male ones in respect of the capacity of vertebral canals, especially in the section between vertebra 6 and 10. Table IV shows how the percentage of average bone mass volume of the vertebrae grew in both sexes and the percentage of vertebral canal capacity of respective cervical vertebrae in the whole cervical spine of the emus of both sexes. These values increased the more the distance to the bird trunk decreased. Table V provides information on changes in the values of vertebral canal capacity indicator for respective cervical vertebrae in relation to the length of their bodies, which indicates changes in the shape of the spinal cord leaving the cranial cavity and running inside them and the cuneate fasciculi that build its beginning. In the whole cervical spine, the greatest value of this ratio is observed in the case of vertebra 17 in both sexes. The results of measurements obtained allowed estimation of the values of correlation coefficients for body weight and the volume of vertebrae and the capacity of their canals. These correlations were weak; whereas those estimated for the volume of vertebrae x the capacity of vertebral canal were medium and non-significant (Table VI). The examined birds were characterized by sexual dimorphism in the length of cervical spine. Female emus had a significantly ($P\leq0.01$) longer neck compared to the male ones (Table VII), as well as the capacity of their vertebral canal was greater ($P\leq0.05$). Estimation of the relative values for the analyzed traits in relation to the whole cervical spine confirms the earlier results (Table VIII).

Table I. Mean values for the metric traits of Emu cervical vertebrae.

Vertebrae	Male emus n	Length of vertebra x	sd	Breadth of vertebra x	sd	Height of vertebra x	sd	Female emus n	Length of vertebra x	sd	Breadth of vertebra x	sd	Height of vertebra x	sd
1	5	4.93	1,11	15.57	1.10	16.00	0.83	6	5.23	0.82	15.7	1.15	16.6	0.89
2	6	26.79	2.61	23.79	2.22	26.94	2.01	8	28.37	1.72	24.4	1.32	26.4	2.31
3	6	24.98[A]	0.94	25.62	0.92	23.86	0.75	7	30.14	2.74	26.7	2.67	24.3	1.62
4	6	29.80	2.83	25.19[a]	1.22	25.92	0.91	10	33.67	4.19	27.2	1.69	24.7	1.36
5	5	34.66[a]	2.88	28.09	1.40	25.14	1.80	10	38.66[a]	3.21	29.6	2.40	25.0	1.67
6	6	36.76[A]	2.10	29.82	1.47	23.33	1.65	10	41.78	2.30	29.8	2.47	24.7	2.39
7	6	38.29[a]	3.54	30.50[a]	1.04	24.76[a]	1.78	10	43.67[a]	3.02	31.7	1.03	27.0	2.37
8	6	41.15[a]	3.43	30.54[a]	1.06	23.40[a]	2.76	10	45.97[a]	1.74	32.0	1.30	29.0	2.75
9	6	43.93[a]	3.71	30.07[a]	1.45	29.69	2.67	10	48.70[a]	2.99	31.9	1.37	31.4	3.25
10	6	46.75	4.02	30.62[a]	1.28	30.63[a]	2.13	10	50.50	3.26	32.7	1.58	32.8	1.92
11	6	48.54	3.67	31.23[a]	1.12	31.52[a]	2.00	10	51.25	3.99	33.4	1.54	34.2	1.95
12	6	49.30[a]	2.49	32.10[A]	1.20	32.45[a]	2.23	10	53.39[a]	2.30	34.6	1.40	36.0	3.21
13	6	51.57	3.76	33.93	1.31	36.08	1.91	10	53.84	2.46	35.2	1.42	36.7	2.25
14	6	53.24	2.93	34.17[a]	2.07	35.57	3.57	10	55.06	2.16	37.4	1.51	38.5	2.62
15	6	53.05[a]	3.13	37.50[a]	2.28	39.89	4.40	9	57.12[a]	2.40	40.2	1.83	40.9	3.22
16	6	52.71	1.76	40.93[a]	3.14	41.23	4.10	10	57.00	1.94	44.6	3.02	43.8	4.63
17	6	52.57[a]	2.36	47.20[a]	5.12	47.22	6.82	10	56.97[a]	2.48	51.3	5.40	49.7	9.38

Explanations: mean values in rows marked with the same letters differ significantly at: a – P≤0.05.

Table II. Mean values of caudal and cranial articular surface breadth and caudal vertebral foramen surface area in Emus males and females.

Vertebrae	Male emus n	Breadth of cranial articular surface x	sd	Breadth of caudal articular surface x	sd	Surface area of vertebral foramen x	sd	Female emus n	Breadth of cranial articular surface x	sd	Breadth of caudal articular surface x	sd	Surface area of vertebral foramen x	sd
1	5	7.33	1.37	10.54	0.87	36.50	2.48	6	7.30	0.87	10.89	1.13	37.08	7.64
2	6	10.36	0.79	7.21	0.54	31.59	4.60	8	9.89	0.57	7.55	0.77	33.72	6.41
3	6	9.15	0.72	8.13	0.45	33.57	5.43	7	10.16	0.97	11.85	3.42	37.09	5.97
4	6	10.36	1.05	13.38	3.02	34.49	5.84	10	12.04	2.50	15.01	3.57	39.45	5.92
5	5	14.58	3.29	18.28	1.74	38.02	4.70	10	17.47	3.46	18.36	2.14	42.34	6.24
6	6	19.80	1.79	19.83	1.18	38.97	7.07	10	20.91	1.64	18.92	1.84	43.30	4.99
7	6	21.32	1.35	18.02	1.16	40.14	7.49	10	20.91	1.82	17.33	1.02	44.48	5.79
8	6	19.89	1.19	16.44	1.26	41.86	7.42	10	19.71	2.27	15.51	1.34	49.70	10.3
9	6	18.59	1.66	15.39	1.09	45.58	6.18	10	18.18	1.83	14.88	1.31	54.45	11.6
10	6	17.58	1.04	15.09	1.07	50.00	5.61	10	17.23	2.29	15.19	1.02	60.31	10.7
11	6	17.38	0.98	15.12	1.20	56.23	11.0	10	18.09	2.21	15.67	1.46	65.67	12.6
12	6	19.78	2.15	15.68	1.51	56.86	10.8	10	19.19	2.17	16.40	1.63	69.05	12.5
13	6	18.60	1.87	17.10	2.17	62.65	12.6	10	19.00	1.27	17.03	2.35	73.14	14.1
14	6	18.26	2.31	17.56	2.08	62.10	7.95	10	20.42	1.00	19.02	1.22	80.68	17.1
15	6	20.53	2.78	19.23	2.17	71.17	10.2	9	21.85	1.69	20.66	0.98	82.73	17.1
16	6	22.03	2.98	20.48	2.14	75.50	13.0	10	23.34	1.60	22.48	1.22	88.55	18.2
17	6	23.73	2.96	22.22	2.09	79.03	15.8	10	25.45	2.09	23.88	1.32	91.44	18.5

Table III. Mean values of vertebral canal length, vertebral canal capacity and vertebral bone mass volume in male and female Emus.

Vertebrae	n	Male emus Length of vertebral		Volume of vertebral		Capacity of vertebral canal		n	Female emus Length of vertebral canal		Volume of vertebral		Capacity of vertebral canal	
		x	sd	x	sd	x	sd		x	sd	x	sd	x	sd
1	5	4.75	1.09	0.924	0.742	0.170	0.030	6	4.82	0.90	0.90	0.228	0.18	0.060
2	6	20.1	1.36	1.550	0.235	0.634	0.099	8	21.20	3.45	1.58	0.498	0.74	0.218
3	6	18.3	1.24	1.884	0.164	0.621	0.136	7	21.92	2.34	1.94	0.533	0.85	0.209
4	6	21.5	1.46	1.997	0.346	0.742[a]	0.122	1	23.99	2.56	2.30	0.580	0.95	0.206
5	5	23.4	2.21	2.213	0.316	0.894	0.149	1	27.39	2.51	2.65	0.508	1.16	0.240
6	6	25.6	1.78	2.776	0.298	1.013[a]	0.211	1	39.94	2.40	3.12	0.476	1.29	0.199
7	6	28.0	1.95	3.346	0.429	1.130[a]	0.239	1	31.64	1.91	3.54	0.735	1.41	0.224
8	6	30.8	2.19	3.770	0.698	1.294[a]	0.260	1	34.07	1.54	4.30	0.839	1.69	0.342
9	6	33.3	2.60	4.248[a]	0.821	1.522[a]	0.250	1	36.15	1.89	5.34	1.124	1.96	0.369
10	6	35.7	2.22	4.707[a]	0.380	1.781	0.185	1	37.68	1.99	6.04	0.947	2.33	0.517
11	6	37.2	2.37	5.440[a]	0.787	2.094	0.425	1	38.60	2.54	6.81	1.223	2.51	0.384
12	6	38.3	2.54	6.156[a]	0.549	2.291	0.441	1	40.43	1.97	7.86	1.476	2.78	0.450
13	6	39.6	2.24	7.098[a]	0.632	2.477	0.511	1	41.21	1.12	8.88	1.485	3.01	0.570
14	6	39.9	2.22	7.809[a]	0.741	2.470[a]	0.260	1	42.05	1.65	9.82	1.675	3.36	0.781
15	6	41.2	2.16	8.815[a]	1.095	2.923	0.377	9	43.46	1.01	11.1	2.010	3.59	0.744
16	6	41.4	1.97	9.844[a]	1.212	3.115	0.476	1	43.92	1.40	12.9	2.237	3.89	0.938
17	6	41.7	2.34	11.34	1.652	3.284	0.623	1	43.83	0.99	14.4	2752	4.01	0.844

Explanations: mean values in rows marked with the same letters differ significantly at: a – P≤0.05; A – P≤0.01.

Table IV. Mean values of vertebral bone mass volume and vertebral canal capacity with their percentages in male and female Emus (differences between sex groups are presented in Table III).

Vertebrae	Mean vertebral bone mass volume and its percentage male emus		female emus		Mean vertebral canal capacity and its percentage male emus		female emus	
	x	%	x	%	x	%	x	%
1	0.924	0.919	0.901	0.531	0.171	0.403	0.182	0.357
2	1.550	1.849	1.587	1.558	0.634	2.241	0.749	1.959
3	1.884	2.248	1.948	1.913	0.621	1.828	0.856	1.961
4	1.997	2.383	2.304	2.262	0.742	2.623	0.951	3.111
5	2.213	2.641	2.658	2.609	0.894	3.158	1.166	3.816
6	2.776	3.313	3.120	3.063	1.013	3.580	1.298	4.239
7	3.346	3.993	3.541	3.477	1.130	3.994	1.410	4.614
8	3.770	4.499	4.306	4.228	1.294	4.574	1.690	5.530
9	4.248	5.070	5.344	5.246	1.522	5.377	1.961	6.417
10	4.707	5.617	6.048	5.937	1.781	6.294	2.339	7.388
11	5.44	6.533	6.812	6.688	2.094	7.401	2.514	8.227
12	6.156	7.347	7.866	7.723	2.291	8.098	2.780	9.095
13	7.098	8.459	8.889	8.727	2.477	8.753	3.011	9.853
14	7.809	9.319	9.827	9.648	2.470	8.731	3.365	9.912
15	8.815	10.521	11.113	10.909	2.923	10.331	3.594	11.759
16	9.844	11.749	12.930	12.694	3.115	11.008	3.894	12.740
17	11.344	13.536	14.470	12.785	3.284	11.604	4.012	13.128

Table V. Mean values of the vertebral canal capacity to vertebral body length ratio for respective cervical vertebrae in male and female Emus.

Vertebrae	Vertebral canal capacity to vertebral body length ratio male emus			female emus		
	n	x	sd	n	x	sd
1	5	3.63	0.28	6	3.71	0.76
2	6	2.39	0.43	7	2.84	0.53
3	6	2.48	0.45	7	2.81	0.48
4	6	2.50	0.40	10	2.82	0.46
5	6	2.58	0.37	10	3.00	0.47
6	6	2.75	0.51	10	3.11	0.45
7	6	2.94	0.52	10	3.23	0.49
8	6	3.15	0.60	10	3.68	0.80
9	6	3.46	0.46	10	4.04	0.83
10	6	3.83	0.50	10	4.51	0.85
11	6	4.34	0.93	10	4.97	1.07
12	6	4.67	0.96	10	5.23	0.99
13	6	4.83	1.02	10	5.59	1.07
14	6	4.65	0.59	9	6.15	1.59
15	6	5.53	75	10	6.27	1.19
16	6	5.91	0.85	10	6.84	1.49
17	6	6.24	1.13	10	7.03	1.37

Table VI. The values of correlation coefficients for body weight and cervical vertebrae volume and capacity in male and female Emus.

Coefficient of correlation	Body weight x cervical vertebrae volume		Body weight x cervical vertebrae capacity		Cervical vertebrae volume x cervical vertebrae capacity	
	male emus	female emus	male emus	female emus	male emus	Female emus
R =	-0.277	0.074	-0.107	-0.263	0.758	0.455

Table VII. The values of bone mass volume, length and capacity for Emu cervical vertebrae.

Trait	Length of cervical spine male emus	female emus	Volume of cervical spine bone mass male emus	female emus	Capacity of cervical spine canal male emus	female emus
x	675.05[A]	722.60[A]	85.58	95.68	28.36[a]	34.53[a]
sd	28.96	35.72	8.40	21.71	3.57	6.08

Explanations: mean values in rows marked with the same letters differ significantly at: a – P≤0.05; A – P≤0.01.

Table VIII. The values of indicators reporting the correlations between cervical spine length and its bone mass volume and canal capacity in male and female Emus.

Indicator	Male emus	Female emus
Mean cervical spine canal capacity x 100 / Mean cervical spine length	4.201	4.778
Mean cervical spine bone mass volume x 100 / Mean cervical spine length	12.677	13.241
Mean cervical spine canal capacity x 100 / Mean cervical spine bone mass	33.138	36.089

DISCUSSION

Calculation of the capacity of vertebral canals of all vertebrae allows the places of vertebral canal narrowing and extensions to be determined. The course of the spinal cord in birds shows many similarities to that in mammals, despite the pronounced species variation. Differences result from the number of vertebrae in the spine and the size of the chest, and the nerve plexuses and vertebral canal extensions and narrowing being present in respective sections of the spine (Goodman & Schein, 1974; Ozmen, 2011). The study carried out did not show any extensions or narrowing within the whole way of the spinal cord through the canals of respective cervical vertebrae of the male and female emus. The breadth and height of respective vertebrae and the surface area of their vertebral foramina in the emus of both sexes had a linear distribution; also the volume of bone mass and the capacity of vertebral canals show that within the whole course of the cervical spine there is no narrowing or extension of the vertebral canal for the spinal cord running in it.

The study carried out and the data collected are a contribution to questions about the values of subsequent vertebrae in the spine of birds belonging to the subclass Palaeognathae. The methods used for measuring the metric traits of cervical vertebrae and the results obtained can also support and complement studies on bone mineral concentration (BMC – insufficient content of minerals in the bone tissue) and bone density (BMD – improper mineral density) in the birds being kept under industrial conditions and seasonally affected by the laying (Kim *et al*., 2006; Dzierzęcka & Charuta, 2010) and exposed particularly during this period to calcium-phosphate imbalance. The use of the hydrostatic method seems to be best to determine the size of vertebrae. Measurement of the volume, replacing several other linear measurements being made even with high precision but not reflecting the real picture of the size of these irregular in shape bone units, may be used in osteometric studies.

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RESUMEN: Este estudio tuvo como objetivo la estimación de los valores de los rasgos métricos básicos de las vértebras cervicales del Emu. El estudio se realizó en vértebras de 6 machos y 10 hembras Emu de catorce años. Las mediciones osteométricas se realizaron con pinzas electrónicas, mientras que el método hidrostático se usó para evaluar la densidad y el volumen de cada vértebra. El sexo de las aves se consideró como una variación. La columna cervical contaba con 17 vértebras. Se encontró dismorfismo en rasgos métricos básicos entre vértebras análogas de ambos sexos. Las vértebras de las hembras se caracterizaron por una longitud, ancho y altura significativamente mayor (P≤0,05 y P≤0,01) a las de los machos. No se encontraron diferencias dismórficas en el volumen de masa ósea para las vértebras 1 a 8, mientras que las vértebras de las hembras 9 a 17 tuvieron un volumen mayor (P≤0,05) en comparación con los machos. Los coeficientes de correlación para el peso corporal, el volumen de la vértebra y la capacidad del canal espinal fueron débiles. La suma de la longitud de los cuerpos vertebrales que determina la longitud del cuello mostró significativamente (P≤0.01) cuellos más largos en las hembras. No se observaron estrechamientos y extensiones del canal vertebral para la médula espinal que se encuentra en toda la columna cervical.

PALABRAS CLAVE: Canal cervical; Vértebra cervical; Método hidrostático; Ratiatae; Palaeognathae; Emu.

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