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A stereological study on calculation of volume values of the cervical spinal segments in ducks

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Abstract: In the present study, volume values of the cervical segments in the spinal cords of adult ducks weighing 3–4 kg were investigated. Volume values of the grey matter and white matter in the cervical segment of the spinal cord of the ducks were examined stereologically. Ten ducks (genus *Anas*) without regarding the sex of the animals. All animals were perfused using 10% formaldehyde. The animals were kept in 10% formaldehyde for 1 week to ensure fixation. The ducks were then dissected. The cervical segments were uncovered by removing the cervical vertebrae. Tissue samples were obtained from each of the cervical spinal segments, and 5-µm-thick cross-sections were obtained from these samples. Sampling was performed at a ratio of 1/250 to obtain 12 cross-sections from each cervical segment of the animals. These sections were stained using the hematoxylin and eosin staining technique. Photos were taken under a microscope. Volume values of whole tissue, grey matter, and white matter were calculated for each cervical segment of the spinal cord of the ducks. Cavalieri's principle was employed for the calculation. As a result, the vertebral column was used as a guide to identify the cervical segments of the spinal cord. The cervical segments were also obtained by dissection without using a decalcification process. It was determined that the number of segments was 15. When mean volume values of the whole cervical segment of the spinal cord in the ducks were evaluated, the highest mean volume was determined as 4.224 mm^3 in segment C15. The cervical spinal segment with the lowest value of white matter was C7 (0.915 mm^3). When the volume values of the grey matter of the cervical segments of the ducks were examined, it was determined that segments C14 and C15 had the highest values, calculated as 0.511 mm^3 and 0.513 mm^3, respectively. It was determined that segments C12, C13, C14, and C15 were involved in the cervical enlargement.

Key words: Cavalieri's principle, cervical segment, duck, spinal cord, stereology, volume

1. Introduction

The duck, belonging to the family Anatidae from the order Anseriformes of the class Aves, is from the genus *Anas*. The present study aimed to stereologically investigate the cervical spinal cord segment of ducks. In poultry, the spinal cord (SC) starts from the foramen magnum, extends to the coccygeal section at the end of the vertebral column, and does not form a cauda equina—it enters into a terminal thread. The spinal cord is thin, narrow, and long in poultry (1). The meninges of the brain—the dura mater, the arachnoid, and the pia mater—also surround the spinal cord (2). In poultry, the spinal cord is examined in 4 parts: cervical (C), thoracic, lumbosacral, and caudal (3). In poultry, the outside area of the spinal cord is formed by white matter (WM), while its inside is formed by grey matter (GM) (2). In cross-section, the grey matter has the appearance of a capital H. The central canal is a visible canal located in the middle of the capital H (4).

Stereology is a discipline that can evaluate and reveal characteristics of 2-dimensional structures in 3 dimensions (5,6). Unbiased methods using stereological techniques are preferred instead of biased methods (7). It has been stated that volume values of every kind of structure can be calculated with Cavalieri's principle by distinguishing the borders, regardless of the relationship of a tissue or biological structure with the surrounding structures (8).

A literature review showed that anatomical and stereological studies have not been done on the cervical segment of the spinal cord in ducks of the genus *Anas*.

The aim of the present study was to determine total volume, grey matter volume, and white matter volume of the cervical spinal segments in adult ducks. The obtained results are presented here as reference values for information sources in the international literature.

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2. Materials and methods

2.1. Animals

A total of 10 adult ducks (*Anas*) of both sexes weighing 3–4 kg were used as the material in the present study. The animals, which were anesthetized by injection of ketamine hydrochloride (50 mg/kg IV) (9), were perfused using the intracardiac method (10) using 10% formaldehyde. The ducks were kept in the formaldehyde pool for the fixation process for 1 week following the perfusion process.

2.2. Removal of the spinal cord

In order to uncover the cervical vertebrae of the fixed ducks, the dissection process began with removal of the soft tissues around the vertebrae. The arcus vertebrae of the cervical vertebrae were then removed. All of the bone tissues were not extirpated because segmentation of the spinal cord was performed with the presence of bone tissue where the spinal cord was located. Tissue samples were obtained from each segment after segmentation (11).

2.3. Sampling method

A pilot study was carried out first to identify the number of animals, the number of cross-sections, and the samples to be used in the study. Since the current studies in the literature state that the relative error of the error coefficient was determined as 10% for stereological studies, the number of animals required for the study was 5 in order to obtain this value (12,13). For this reason, 5 adult ducks were used in the pilot study. In calculation of the coefficient of error for the sampling method in the present study, the total variant square root/total number of points was taken as a basis. SHTEREOM 1.0 software was used to calculate the number of cross-sections (8,14).

Tissues dissected from the spinal cord cervical segments of the ducks were subjected to tissue processing to conduct the pilot study. All tissues were embedded vertically in paraffin in the same direction following the tissue processing. Transverse sections of 5 µm thick were obtained from these tissues, embedded, and blocked in paraffin via rotary microtome (Leica RM 21, 35, Nussloch, Germany) until the tissue was finished by using a random systematic sampling method. These sections were transferred onto microscope slides. The first step of deparaffinization was ensured by keeping them in a drying oven. The deparaffinization process was then finished by passing them through a series of xylol and alcohol. The sections were stained using hematoxylin and eosin and covered with coverslips (11,15). Cross-sections were sampled by performing the stepping at a ratio of 1/250 to obtain 12 cross-sections from each segment of the animals. One out of the first 250 cross-sections was randomly chosen for systematic random sampling. Every 250th cross-section was then placed on the microscope slide after counting. Results obtained from the pilot study were included in the original study, as the values from the pilot study were appropriate for the present study.

2.4. Image analysis

Stereological stepping was needed to examine the duck spinal cords, because the spinal cord has a large structure in poultry at 4× magnification. Stereological stepping was done using a motorized stage and photographs were taken. Hence, measurements were obtained from 4× magnification photos. Point grid and 4× magnification were used for calculating the area and then volume. SHTEREOM 1.0 software was used to complete the calculations (Figure 1). In addition to the program, Cavalieri's principle was applied as the calculation method (16). Total volumes of 15 cervical segments of the spinal cord, total volume of grey matter, and total volume of white matter of the ducks were calculated (Figures 2 and 3). The number of points was used because the ratio of points could be included as the volume in the calculation of volume values (7,17).

In this study, a one-way ANOVA test was applied for the comparison between the segments. SPSS was used for statistical analysis.

3. Results

When mean volume values of the entire cervical segment of the spinal cord in the ducks were evaluated, the highest mean volume was 4.224 mm^3 in segment C15. This value, which was 3.735 mm^3 in segment C1, decreased to 2.959 mm^3 by showing a distinct decline up to segment C5. This value increased to 3.126 mm^3 in segment C8. The total volume value of the cervical segment, which continued to increase in segments C9, C10, and C11, was 3.701 mm^3 in segment C12. While this value was 4.139 mm^3 in segment C13, it was 4.218 mm^3 in segment C14 and 4.224 mm^3 in segment C15 (Table 1).

When volume values of the white matter in the spinal cord cervical segments of the ducks given in Table 2 were examined, the highest value belonged to segment C5 (1.192 mm^3), while the lowest value belonged to segment C7 (0.915 mm^3). While volume of the white matter was 1.042 mm^3 in segment C1, it was 1.054 mm^3 in segment C2. This value was 1.240 mm^3 in segment C12, decreasing to 1.171 mm^3 in segment C13. It decreased further for segments C14 and C15, with values of 0.994 mm^3 and 0.991 mm^3, respectively.

When volume values of the grey matter in the spinal cord cervical segments of the ducks were examined, it was determined that segments C14 and C15 had the highest values, calculated as 0.511 mm^3 and 0.513 mm^3, respectively. Mean volume value of the grey matter of segment C1 was 0.425 mm^3; this value decreased until segment C9. Mean volume value of the grey matter was 0.319 mm^3 in segment C10, and it increased up to segment C15 (Table 2).

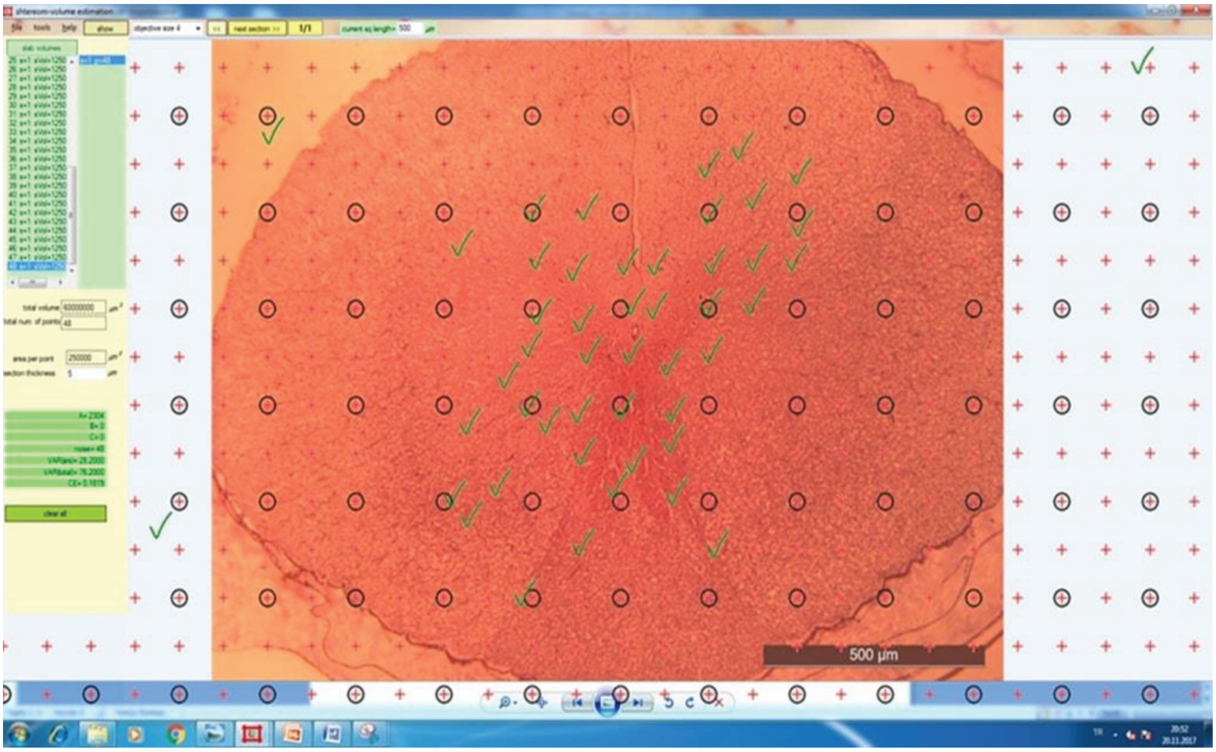

Figure 1. Image analysis by SHTEREOM 1.0 program.

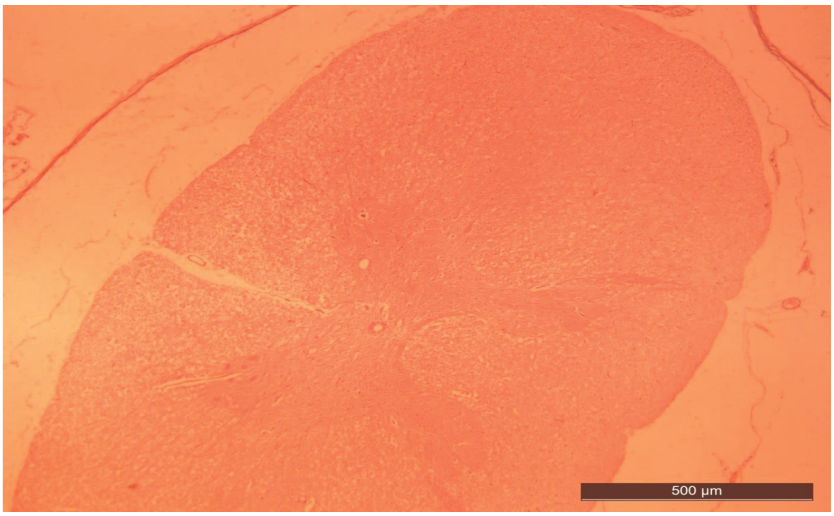

Figure 2. The cervical spinal segment of the duck at 4× magnification (hematoxylin and eosin).

As the grey matter/white matter volume ratios of the cervical segments of the ducks in Table 2 were examined, the highest mean volume ratio was found in segment C15; this value was calculated as 0.941%. The lowest grey matter/white matter volume ratio was 0.300%, which belonged to segment C5. While the GM/WM volume ratio was 0.481% in segment C1, this volume ratio decreased until segment C5. This value started to increase at segment C6 and showed a significant increase from segment C13.

When the results of the white matter/spinal cord volume ratio in the cervical spinal segments of the ducks were examined, the highest value was observed as 0.367% in segment C8. The WM/SC volume ratio of segment C1 was calculated as 0.280%. This value was 0.290% for segments C2 and C3. The WM/SC volume ratio was seen to increase until segment C8. This value was 0.322% in

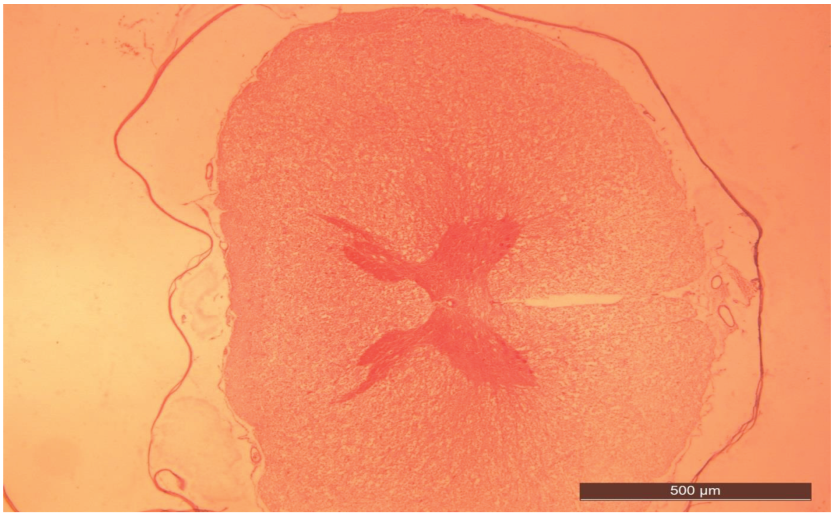

Figure 3. The cervical spinal segment of the duck at 4× magnification (hematoxylin and eosin).

segment C9; it then decreased. The lowest white matter/spinal cord volume ratio belonged to segments C14 and C15; this ratio was determined as 0.240% (Table 1).

The values given in Table 1 indicate that the lowest grey matter/spinal cord volume ratio of the cervical spinal segments of the ducks was 0.090%, which belonged to segment C5. The highest grey matter/spinal cord volume ratio was observed in segments C14 and C15 (0.120%). The GM/SC volume ratio of segment C1 was 0.119%. This ratio decreased until segments C9 and C10. There was a significant increase in segment C13, the value of which was calculated as 0.115%.

In addition, it was determined that while the grey matter volume values were higher in segments C14 and C15 compared to the other segments, the white matter volume ratios were lower in these segments than in the other segments. While the white matter volume values were high for segments C1 and C2, volume values of grey matter were low in segments C1 and C2 (Table 2).

Values of the noise and coefficient of error (CE) corresponding to whole tissue (Table 3), white matter (Table 4), and grey matter (Table 5) belonging to the cervical spinal segments of the ducks were calculated. The noise and coefficient of error (CE) values were calculated and volume calculation was performed with the SHTEREOM program.

It was determined that the average CE of whole tissue of the cervical segments was 0.0334. The average CE of white matter of the cervical segments was 0.0441. It was determined that the average CE of grey matter of the cervical segments was 0.0433 in this study.

The mean number of points for whole tissue was calculated as follows: C1 = 1023, C2 = 978, C3 = 936, C4 = 924, C5 = 919, C6 = 918, C7 = 819, C8 = 865, C9 = 904, C10 = 913, C11 = 943, C12 = 1024, C13 = 1145, C14 = 1167, C15 = 1154. It was determined that the highest mean number of points for the whole tissue of the cervical spinal segments belonged to the C14 segment, and the lowest mean number of points belonged to segment C7.

The mean number of points for the white matter of the cervical spinal segments was calculated as follows: C1 = 491, C2 = 568, C3 = 513, C4 = 524, C5 = 522, C6 = 524, C7 = 499, C8 = 507, C9 = 520, C10 = 521, C11 = 532, C12 = 559, C13 = 585, C14 = 563, C15 = 559. The highest mean number of points for the white matter of the cervical spinal segments belonged to segment C13. The lowest mean number of points belonged to segment C1.

The mean number of points for the grey matter of the cervical spinal cord was calculated as follows: C1 = 667, C2 = 555, C3 = 501, C4 = 513, C5 = 484, C6 = 460, C7 = 463, C8 = 467, C9 = 518, C10 = 522, C11 = 542, C12 = 508, C13 = 726, C14 = 826, C15 = 856. The highest mean number of points for the grey matter of the cervical spinal segments belonged to segment C15. The lowest mean number of points belonged to segment C6.

3.1. Data analysis results

When the total volume value of the cervical segment in ducks was examined statistically, no difference was found between segments C1, C2, and C12. While no difference was found between segments C3 and C11, the difference between these segments and the other segments was significant. There was no difference between segments C4, C5, C6, C7, C8, C9, and C10; however, a difference was observed between the other segments and these segments. While there was no difference between segments C14 and C15, the difference between these segments and the

Table 1. Volume values of the cervical spinal cord of ducks (VVSC) (mm³); volume ratios of the white matter/spinal cord in the cervical spinal segments of ducks (WM/SC) (%); volume ratios of the grey matter/spinal cord in the cervical spinal segments of ducks (GM/SC) (%).

Number of segment			Number of animal										
			D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	Mean
	C1	VVSC	2.666	2.113	4.537	6.180	3.952	3.103	4.092	4.443	4.009	2.250	3.735
		WM/SC	0.195	0.396	0.379	0.250	0.399	0.203	0.149	0.371	0.159	0.306	0.280
		GM/SC	0.178	0.174	0.097	0.126	0.107	0.118	0.093	0.078	0.089	0.130	0.119
	C2	VVSC	2.210	2.185	4.663	4.396	3.691	3.327	3.995	4.508	3.395	2.973	3.534
		WM/SC	0.271	0.300	0.365	0.373	0.442	0.197	0.158	0.366	0.202	0.228	0.290
		GM/SC	0.161	0.157	0.075	0.067	0.098	0.101	0.092	0.076	0.091	0.107	0.102
	C3	VVSC	2.033	3.449	3.272	4.186	4.223	2.922	3.764	3.944	3.446	2.572	3.381
		WM/SC	0.329	0.481	0.222	0.406	0.383	0.236	0.176	0.178	0.211	0.283	0.290
		GM/SC	0.159	0.098	0.090	0.062	0.088	0.104	0.086	0.074	0.079	0.101	0.094
	C4	VVSC	2.174	3.146	4.176	4.414	4.067	3.045	3.778	3.402	2.962	2.239	3.340
		WM/C	0.321	0.232	0.415	0.389	0.415	0.229	0.187	0.199	0.246	0.281	0.291
		GM/SC	0.135	0.084	0.068	0.060	0.074	0.096	0.076	0.094	0.088	0.164	0.093
	C5	VVSC	3.211	2.333	4.071	3.793	3.598	3.395	3.348	2.951	3.258	3.254	3.321
		WM/SC	0.538	0.312	0.400	0.448	0.469	0.500	0.206	0.237	0.222	0.193	0.352
		GM/SC	0.082	0.111	0.072	0.088	0.085	0.086	0.091	0.100	0.084	0.101	0.090
	C6	VVSC	3.301	3.301	3.782	4.002	3.598	2.904	3.363	2.474	3.413	3.038	3.318
		WM/SC	0.454	0.529	0.457	0.439	0.192	0.248	0.210	0.295	0.210	0.210	0.324
		GM/SC	0.080	0.076	0.066	0.065	0.084	0.095	0.087	0.106	0.082	0.120	0.086
	C7	VVSC	1.762	3.016	3.142	4.027	3.034	2.691	3.009	2.727	3.074	3.103	2.959
		WM/SC	0.400	0.232	0.554	0.432	0.214	0.271	0.243	0.272	0.238	0.219	0.307
		GM/SC	0.167	0.094	0.082	0.064	0.115	0.102	0.088	0.094	0.087	0.102	0.099
	C8	VVSC	1.491	2.987	3.533	3.579	3.807	2.694	3.850	3.608	2.828	2.882	3.126
		WM/SC	0.489	0.585	0.221	0.481	0.177	0.280	0.438	0.471	0.248	0.225	0.361
		GM/SC	0.180	0.084	0.078	0.070	0.082	0.090	0.080	0.082	0.105	0.121	0.097
	C9	VVSC	2.279	3.356	3.525	3.464	4.197	3.088	3.424	2.651	3.366	3.312	3.266
		WM/SC	0.252	0.510	0.495	0.483	0.397	0.215	0.210	0.273	0.200	0.190	0.322
		GM/SC	0.183	0.083	0.089	0.072	0.078	0.105	0.082	0.105	0.093	0.112	0.100
	C10	VVSC	2.120	3.468	3.421	3.937	4.367	3.074	3.858	2.485	3.142	3.139	3.301
		WM/SC	0.271	0.196	0.512	0.431	0.377	0.536	0.445	0.281	0.222	0.201	0.347
		GM/SC	0.168	0.090	0.087	0.067	0.085	0.113	0.072	0.117	0.094	0.115	0.100
	C11	VVSC	2.297	2.647	4.432	4.251	4.284	4.085	3.879	1.990	3.410	2.814	3.409
		WM/SC	0.282	0.259	0.388	0.395	0.373	0.159	0.171	0.371	0.211	0.195	0.280
		GM/SC	0.142	0.118	0.070	0.065	0.095	0.085	0.087	0.130	0.081	0.159	0.103
	C12	VVSC	2.275	3.388	4.443	5.238	4.201	3.215	4.074	3.504	3.551	3.121	3.701
		WM/SC	0.274	0.464	0.379	0.318	0.383	0.518	0.157	0.456	0.197	0.203	0.334
		GM/SC	0.166	0.074	0.074	0.058	0.085	0.100	0.089	0.114	0.083	0.118	0.937
	C13	VVSC	2.286	3.908	5.115	4.974	4.157	4.074	4.269	4.064	4.074	4.468	4.139
		WM/SC	0.601	0.415	0.328	0.319	0.155	0.170	0.385	0.398	0.157	0.045	0.297
		GM/SC	0.273	0.109	0.080	0.063	0.085	0.107	0.081	0.092	0.086	0.178	0.115
	C14	VVSC	2.933	4.316	2.709	5.191	5.328	3.363	5.631	4.009	4.111	4.584	4.218
		WM/SC	0.533	0.375	0.177	0.332	0.248	0.208	0.213	0.129	0.153	0.039	0.240
		GM/SC	0.157	0.087	0.098	0.098	0.124	0.108	0.141	0.120	0.090	0.178	0.120
	C15	VVSC	2.931	4.315	2.712	5.195	5.325	3.367	5.700	4.002	4.120	4.580	4.224
		WM/SC	0.532	0.374	0.175	0.333	0.247	0.210	0.211	0.124	0.150	0.038	0.240
		GM/SC	0.158	0.086	0.096	0.097	0.123	0.111	0.137	0.123	0.091	0.182	0.120

Table 2. Volume values of the white matter of the cervical spinal segments of ducks (VVWM) (mm^3); volume values of the grey matter of the cervical spinal segments of ducks (VVGM) (mm^3); volume ratios of the grey matter/white matter in the cervical spinal segments of ducks (GM/WM) (%).

Number of segment			Number of animal										
			D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	Mean
	C1	VVWM	0.520	0.837	1.720	1.550	1.579	0.631	0.610	1.650	0.640	0.690	1.042
		VVGM	0.475	0.368	0.441	0.784	0.425	0.369	0.384	0.350	0.360	0.307	0.426
		GM/WM	0.913	0.439	0.256	0.505	0.269	0.584	0.629	0.212	0.562	0.444	0.481
	C2	VVWM	0.600	0.656	1.703	1.640	1.635	0.657	0.635	1.650	0.689	0.680	1.054
		VVGM	0.357	0.344	0.354	0.297	0.365	0.338	0.368	0.344	0.311	0.319	0.339
		GM/WM	0.595	0.524	0.207	0.181	0.223	0.514	0.579	0.208	0.451	0.469	0.395
	C3	VVWM	0.670	1.659	0.727	1.700	1.620	0.690	0.665	0.703	0.730	0.730	0.989
		VVGM	0.324	0.341	0.297	0.263	0.376	0.306	0.327	0.294	0.274	0.262	0.306
		GM/WM	0.483	0.517	0.408	0.154	0.232	0.443	0.491	0.418	0.375	0.358	0.387
	C4	VVWM	0.700	0.730	1.735	1.720	1.690	0.700	0.710	0.680	0.730	0.630	1.002
		VVGM	0.294	0.265	0.286	0.265	0.303	0.294	0.289	0.320	0.263	0.368	0.294
		GM/WM	0.420	0.363	0.164	0.154	0.179	0.420	0.407	0.470	0.360	0.584	0.352
	C5	VVWM	1.730	0.730	1.630	1.700	1.690	1.700	0.692	0.700	0.725	0.631	1.192
		VVGM	0.265	0.259	0.296	0.337	0.307	0.294	0.308	0.297	0.275	0.329	0.296
		GM/WM	0.153	0.354	0.181	0.198	0.181	0.172	0.445	0.424	0.379	0.521	0.300
	C6	VVWM	1.500	1.748	1.730	1.760	0.692	0.723	0.707	0.730	0.720	0.638	1.094
		VVGM	0.295	0.252	0.251	0.263	0.303	0.277	0.293	0.262	0.283	0.366	0.284
		GM/WM	0.176	0.144	0.145	0.149	0.437	0.383	0.414	0.358	0.393	0.573	0.317
	C7	VVWM	0.705	0.700	1.741	1.740	0.650	0.730	0.733	0.743	0.732	0.680	0.915
		VVGM	0.265	0.286	0.260	0.259	0.350	0.276	0.267	0.257	0.268	0.317	0.280
		GM/WM	0.418	0.408	0.149	0.148	0.538	0.378	0.364	0.345	0.366	0.466	0.358
	C8	VVWM	0.730	1.750	0.748	1.724	0.675	0.755	1.690	1.700	0.702	0.650	1.112
		VVGM	0.269	0.251	0.276	0.252	0.314	0.245	0.308	0.296	0.298	0.349	0.285
		GM/WM	0.368	0.143	0.368	0.146	0.465	0.324	0.182	0.174	0.424	0.536	0.313
	C9	VVWM	0.575	1.713	1.748	1.675	1.670	0.665	0.720	0.725	0.674	0.630	1.079
		VVGM	0.418	0.281	0.314	0.252	0.330	0.325	0.284	0.280	0.316	0.372	0.317
		GM/WM	0.726	0.164	0.179	0.150	0.197	0.488	0.394	0.386	0.468	0.590	0.374
	C10	VVWM	0.575	0.680	1.753	1.700	1.650	1.650	1.720	0.700	0.700	0.635	1.176
		VVGM	0.358	0.314	0.300	0.267	0.375	0.350	0.279	0.292	0.298	0.361	0.319
		GM/WM	0.622	0.461	0.171	0.157	0.227	0.212	0.162	0.417	0.425	0.568	0.342
	C11	VVWM	0.650	0.686	1.720	1.683	1.589	0.650	0.665	0.740	0.720	0.550	0.965
		VVGM	0.328	0.314	0.314	0.278	0.411	0.350	0.338	0.260	0.277	0.448	0.331
		GM/WM	0.504	0.457	0.182	0.165	0.258	0.538	0.508	0.351	0.384	0.814	0.416
	C12	VVWM	0.625	1.573	1.685	1.670	1.610	1.667	0.640	1.600	0.700	0.635	1.240
		VVGM	0.378	0.254	0.330	0.308	0.359	0.323	0.365	0.400	0.297	0.370	0.338
		GM/WM	0.604	0.161	0.195	0.184	0.222	0.193	0.570	0.250	0.424	0.582	0.338
	C13	VVWM	1.374	1.625	1.680	1.590	0.645	0.694	1.645	1.620	0.640	0.203	1.171
		VVGM	0.626	0.427	0.410	0.314	0.355	0.438	0.346	0.377	0.354	0.797	0.444
		GM/WM	0.455	0.262	0.244	0.197	0.550	0.631	0.210	0.232	0.553	3.926	0.726
	C14	VVWM	1.565	1.620	0.480	1.725	1.325	0.700	1.202	0.520	0.630	0.180	0.994
		VVGM	0.462	0.377	0.268	0.513	0.662	0.366	0.798	0.482	0.371	0.820	0.511
		GM/WM	0.295	0.232	0.558	0.297	0.499	0.522	0.663	0.926	0.558	4.555	0.910
	C15	VVWM	1.560	1.618	0.475	1.730	1.320	0.710	1.208	0.500	0.620	0.175	0.991
		VVGM	0.465	0.375	0.262	0.509	0.658	0.375	0.785	0.495	0.375	0.835	0.513
		GM/WM	0.298	0.231	0.551	0.294	0.498	0.528	0.649	0.990	0.604	4.771	0.941

Table 3. Coefficient of error (CE) and noise values of the whole cervical spinal segments of ducks.

Number of animal		Number of segment															
		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	Mean
D1	Noise	738	612	563	602	889	914	488	413	631	587	636	630	633	812	820	665
	CE	0.0372	0.0409	0.0429	0.0412	0.0342	0.0337	0.0455	0.0496	0.0402	0.0419	0.0403	0.0403	0.0401	0.0358	0.0349	0.0399
D2	Noise	585	605	955	871	646	914	835	827	929	960	733	938	1082	1195	1190	884
	CE	0.0417	0.0414	0.0332	0.0347	0.0402	0.0338	0.0350	0.0354	0.0335	0.0332	0.0376	0.0336	0.0330	0.0294	0.0287	0.0350
D3	Noise	1256	1291	906	1156	1127	1047	870	978	976	947	1227	1230	1416	750	820	1066
	CE	0.0289	0.0285	0.0341	0.0301	0.0303	0.0319	0.0346	0.0329	0.0326	0.0332	0.0293	0.0296	0.0276	0.0385	0.0387	0.0321
D4	Noise	1711	1217	1159	1222	1050	1108	1115	991	959	1090	1177	1450	1377	1437	1425	1233
	CE	0.0252	0.0293	0.0303	0.0295	0.0316	0.0310	0.0310	0.0329	0.0337	0.0312	0.0296	0.0271	0.0281	0.0276	0.0268	0.0297
D5	Noise	1094	1022	1169	1126	996	996	840	1054	1162	1209	1186	1163	1151	1475	1468	1141
	CE	0.0308	0.0320	0.0301	0.0305	0.0323	0.0322	0.0352	0.0317	0.0302	0.0297	0.0296	0.0301	0.0300	0.0270	0.0265	0.0305
D6	Noise	859	921	809	843	940	804	745	746	855	851	1131	890	1128	931	925	892
	CE	0.0348	0.0336	0.0357	0.0349	0.0333	0.0361	0.0370	0.0369	0.0348	0.0348	0.0307	0.0342	0.0309	0.0333	0.0327	0.0342
D7	Noise	1133	1106	1042	1046	927	931	833	1066	948	1068	1074	1128	1182	1559	1453	1100
	CE	0.0304	0.0310	0.0317	0.0316	0.0337	0.0333	0.0354	0.0317	0.0335	0.0314	0.0313	0.0307	0.0298	0.0259	0.0256	0.0311
D8	Noise	1230	1248	1092	942	817	685	755	999	734	688	551	970	1125	1110	1050	933
	CE	0.0293	0.0291	0.0313	0.0334	0.0352	0.0391	0.0370	0.0323	0.0375	0.0388	0.0433	0.0329	0.0311	0.0308	0.0301	0.0341
D9	Noise	1005	940	954	820	902	945	851	783	932	870	944	983	1128	1138	1120	954
	CE	0.0322	0.0334	0.0329	0.0355	0.0339	0.0332	0.0348	0.0360	0.0336	0.0348	0.0333	0.0327	0.0304	0.0303	0.0301	0.0331
D10	Noise	623	823	712	620	901	841	859	798	917	869	779	864	1237	1269	1275	892
	CE	0.0406	0.0353	0.0378	0.0407	0.0341	0.0364	0.0347	0.0357	0.0336	0.0347	0.0365	0.0346	0.0293	0.0287	0.0281	0.0347

Table 4. Coefficient of error (CE) and noise values of white matter of the cervical spinal segments of ducks.

Number of animal		Number of segment															
		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	Mean
D1	Noise	490	720	425	497	657	620	429	412	528	485	484	514	522	564	572	485
	CE	0.0455	0.0464	0.0491	0.0453	0.0396	0.0405	0.0484	0.0499	0.0438	0.0457	0.0459	0.0444	0.0440	0.0423	0.0418	0.0448
D2	Noise	489	522	576	543	460	586	515	566	577	546	465	576	635	636	639	555
	CE	0.0456	0.0443	0.0421	0.0432	0.0470	0.0417	0.0443	0.0426	0.0421	0.0437	0.0468	0.0422	0.0401	0.0400	0.0398	0.0430
D3	Noise	684	686	509	602	570	574	578	532	570	565	654	637	689	438	425	581
	CE	0.0388	0.0387	0.0452	0.0411	0.0421	0.0425	0.0421	0.0438	0.0422	0.0423	0.0397	0.0403	0.0390	0.0488	0.0491	0.0424
D4	Noise	673	642	658	703	652	679	687	636	569	642	644	759	724	623	615	660
	CE	0.0395	0.0400	0.0395	0.0387	0.0395	0.0390	0.0388	0.0404	0.0429	0.0403	0.0396	0.0370	0.0376	0.0409	0.0413	0.0397
D5	Noise	601	590	581	604	557	498	495	531	625	649	655	605	544	638	642	588
	CE	0.0412	0.0418	0.0417	0.0412	0.0427	0.0451	0.0453	0.0438	0.0404	0.0397	0.0394	0.0412	0.0448	0.0401	0.0398	0.0419
D6	Noise	501	551	458	452	559	484	459	428	489	517	571	490	558	509	492	501
	CE	0.0451	0.0429	0.0472	0.0472	0.0430	0.0458	0.0468	0.0488	0.0455	0.0445	0.0424	0.0457	0.0427	0.0445	0.0451	0.0451
D7	Noise	483	494	553	497	460	473	515	592	518	560	542	540	566	758	762	554
	CE	0.0461	0.0455	0.0429	0.0452	0.0472	0.0469	0.0443	0.0415	0.0453	0.0429	0.0433	0.0436	0.0424	0.0365	0.0360	0.0433
D8	Noise	565	565	523	524	433	419	449	561	459	416	425	565	612	515	512	503
	CE	0.0424	0.0426	0.0441	0.0443	0.0481	0.0499	0.0475	0.0425	0.0471	0.0494	0.0496	0.0426	0.0409	0.0447	0.0450	0.0454
D9	Noise	431	448	440	416	431	471	425	407	434	422	455	496	505	467	458	447
	CE	0.0484	0.0476	0.0478	0.0492	0.0485	0.0463	0.0488	0.0498	0.0482	0.0491	0.0476	0.0452	0.0449	0.0466	0.0468	0.0477
D10	Noise	415	467	412	409	448	443	439	410	438	412	429	417	497	486	482	440
	CE	0.0499	0.0466	0.0497	0.0498	0.0475	0.0478	0.0479	0.0495	0.0480	0.0497	0.0487	0.0498	0.0450	0.0460	0.0465	0.0482

Table 5. Coefficient of error (CE) and noise values of grey matter of the cervical spinal segments of ducks.

Number of animal			Number of segment															
			C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	Mean
	D1	Noise	490	583	529	800	433	434	482	440	683	586	537	618	1023	755	763	562
		CE	0.0365	0.0420	0.0440	0.0463	0.0484	0.0485	0.0458	0.0478	0.0389	0.0417	0.0436	0.0405	0.0321	0.0370	0.0392	0.0422
	D2	Noise	601	563	558	434	423	413	467	411	459	513	513	415	698	617	625	514
		CE	0.0410	0.0425	0.0426	0.0484	0.0497	0.0494	0.0467	0.0498	0.0470	0.0446	0.0446	0.0497	0.0382	0.0410	0.0426	0.0452
	D3	Noise	720	578	486	467	484	411	426	451	513	490	513	590	671	438	420	475
		CE	0.0376	0.0420	0.0458	0.0467	0.0457	0.0496	0.0486	0.0476	0.0444	0.0457	0.0445	0.0435	0.0393	0.0484	0.0498	0.0453
	D4	Noise	1280	485	431	434	551	430	424	412	413	437	454	504	513	838	923	569
		CE	0.0292	0.0458	0.0486	0.0481	0.0428	0.0487	0.0491	0.0499	0.0498	0.0482	0.0470	0.0451	0.0445	0.0354	0.0347	0.0445
	D5	Noise	694	596	614	496	502	496	572	514	540	613	672	587	581	1082	1115	645
		CE	0.0381	0.0413	0.0410	0.0452	0.0449	0.0452	0.0423	0.0445	0.0433	0.0406	0.0391	0.0420	0.0418	0.0315	0.0309	0.0408
	D6	Noise	604	552	501	480	480	453	451	401	532	573	573	528	716	500	450	520
		CE	0.0414	0.0430	0.0451	0.0459	0.0460	0.0474	0.0475	0.0501	0.0440	0.0422	0.0422	0.0439	0.0380	0.0450	0.0472	0.0446
	D7	Noise	627	601	535	473	503	479	437	504	465	456	553	597	566	1303	1325	628
		CE	0.0400	0.0412	0.0439	0.0463	0.0449	0.0463	0.0484	0.0449	0.0446	0.0470	0.0430	0.0412	0.0425	0.0282	0.0271	0.0420
	D8	Noise	572	563	481	523	485	429	420	484	458	477	426	654	616	788	821	521
		CE	0.0421	0.0426	0.0459	0.0440	0.0459	0.0486	0.0492	0.0459	0.0471	0.0462	0.0498	0.0398	0.0412	0.0363	0.0355	0.0440
	D9	Noise	589	508	448	430	450	463	439	488	516	488	453	486	579	607	713	510
		CE	0.0418	0.0448	0.0475	0.0486	0.0474	0.0467	0.0479	0.0456	0.0442	0.0457	0.0473	0.0458	0.0419	0.0409	0.0392	0.0450
	D10	Noise	502	521	428	601	538	599	519	571	608	591	732	605	1302	1339	1412	725
		CE	0.0449	0.0442	0.0486	0.0413	0.0434	0.0412	0.0442	0.0422	0.0409	0.0415	0.0379	0.0424	0.0295	0.0281	0.0272	0.0398

other segments was significant. When the volume of the white matter in the cervical segments of the ducks was examined statistically, no difference was found between the segments. When the volume of the grey matter in the cervical segments of the ducks was evaluated statistically, there was no difference between segments C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, and C12. The difference between the other segments and these segments was significant. The difference between segments C1 and C13 and all of the other segments was significant. While there was no difference between segments C14 and C15, the difference between these segments and the other segments was significant. No difference was determined between the cervical segments of the ducks in terms of GM/WM, WM/SC, and GM/SC volume ratios (Table 6).

4. Discussion

It has been reported that the number of cervical vertebrae is 17 in goose, 14 in chicken, 12 in pigeon, and 14 in duck (3,18,19). Cakmak et al. (11) stated in their study that the number of cervical vertebrae in quails was 12. The number of cervical vertebrae was determined to be 14 in duck in the present study. A study conducted on the spinal cords of duck, chicken, and pigeon revealed that the number of cervical spinal segments was 15 in chicken and 13 in pigeon and duck (20). A stereological study on the cervical segments of quails revealed that the number of cervical spinal segments was 13 (11). In the present study conducted on the cervical spinal segments of ducks, it was determined that the number of segments was 15.

In one study, the spinal cord was segmented along with the vertebral column. The vertebrae were then decalcified (21). In the study conducted by Bolat (22) on Leghorn chicken it was reported that decalcification could be applied to the spinal cord as a whole without segmenting before decalcification. In the present study, the spinal cord was uncovered after the arcus vertebrae of the vertebral column in the spinal cord were removed. The vertebral column was used as a guide to identify the cervical segments of the spinal cord. The cervical segments were also obtained by dissection without using decalcification in the present study.

A study conducted on the cervical segments of the duck and the chicken suggested that the area value of segment C4 was larger compared to the other segments, and the white matter/grey matter ratio of segment C1 was lower than area ratio values of the other segments (20). In the present study, the highest volume value of the cervical segment in duck belonged to segment C15. This volume value obtained in the present study was not the same as the finding of segment C4 having the maximum area in the study by Hazıroğlu et al. (20), conducted on area values of cervical segments in duck.

In the study conducted by Bolat (22) on poultry, it was stated that the cervical enlargement was formed by segments C13, C14, C15, T1, and T2. A study conducted on the cervical segments of quails reported that segments C10, C11, C12, and C13 were involved in the formation of the cervical enlargement (11). Hazıroğlu et al. (20) stated in their study that the cervical enlargement was formed by

Table 6. Data analysis.

	Duck cer. SC volume	Duck cer. WM volume	Duck cer. GM volume	Duck cer. GM/WM ratio	Duck cer. WM/SC ratio	Duck cer. GM/SC ratio
C1	3.735 ± 0.391^{abc}	1.043 ± 0.161	0.426 ± 0.043^{b}	0.481 ± 0.066	0.281 ± 0.032	0.119 ± 0.011
C2	3.534 ± 0.282^{abc}	1.055 ± 0.164	0.340 ± 0.007^{c}	0.395 ± 0.054	0.290 ± 0.030	0.103 ± 0.010
C3	3.381 ± 0.224^{bc}	0.989 ± 0.147	0.306 ± 0.012^{c}	0.388 ± 0.037	0.291 ± 0.033	0.094 ± 0.008
C4	3.340 ± 0.245^{c}	1.003 ± 0.156	0.295 ± 0.010^{c}	0.352 ± 0.045	0.291 ± 0.028	0.094 ± 0.010
C5	3.321 ± 0.149^{c}	1.193 ± 0.166	0.297 ± 0.008^{c}	0.301 ± 0.044	0.353 ± 0.042	0.090 ± 0.004
C6	3.318 ± 0.139^{c}	1.095 ± 0.162	0.285 ± 0.011^{c}	0.317 ± 0.048	0.324 ± 0.041	0.086 ± 0.005
C7	2.959 ± 0.176^{c}	0.915 ± 0.138	0.281 ± 0.010^{c}	0.358 ± 0.039	0.308 ± 0.036	0.100 ± 0.009
C8	3.126 ± 0.227^{c}	1.112 ± 0.165	0.286 ± 0.011^{c}	0.313 ± 0.045	0.362 ± 0.046	0.097 ± 0.010
C9	3.266 ± 0.163^{c}	1.080 ± 0.170	0.317 ± 0.015^{c}	0.374 ± 0.063	0.323 ± 0.042	0.100 ± 0.010
C10	3.301 ± 0.212^{c}	1.176 ± 0.173	0.319 ± 0.012^{c}	0.342 ± 0.056	0.347 ± 0.041	0.101 ± 0.009
C11	3.409 ± 0.286^{bc}	0.965 ± 0.154	0.332 ± 0.019^{c}	0.416 ± 0.061	0.280 ± 0.030	0.103 ± 0.010
C12	3.701 ± 0.260^{abc}	1.241 ± 0.161	0.338 ± 0.014^{c}	0.339 ± 0.059	0.335 ± 0.040	0.096 ± 0.010
C13	4.139 ± 0.242^{ab}	1.172 ± 0.177	0.444 ± 0.048^{ab}	0.726 ± 0.359	0.297 ± 0.052	0.115 ± 0.020
C14	4.218 ± 0.317^{a}	0.995 ± 0.176	0.512 ± 0.060^{a}	0.911 ± 0.410	0.241 ± 0.045	0.120 ± 0.010
C15	4.224 ± 0.315^{a}	0.991 ± 0.173	0.513 ± 0.060^{a}	0.941 ± 0.400	0.240 ± 0.043	0.120 ± 0.010
P-value	0.018	0.982	0.000	0.222	0.722	0.459

segments C13, C14, C15, T1, and T2 in chicken; by segments C12, C13, C14, C15, T1, and T2 in duck; and by segments C11, C12, C13, T1, and T2 in pigeon. In the present study, it was determined that segments C12, C13, C14, and C15 were involved in the cervical enlargement. The segments involved in formation of the cervical enlargement in the study conducted by Hazıroğlu et al. (20) on the cervical segments of duck were compatible with the segments involved in the cervical enlargement area in the present study.

Based on the results obtained from the present study, the whole volume ratios were high in segments C12, C13, C14, and C15 among the cervical segments of the ducks, which suggested that these segments were involved in the formation of the cervical enlargement area in duck.

Consequently, the whole segment, the grey matter, and the white matter differed between segments in terms of volume values of the cervical spinal segments, and these differences also revealed statistical significance.

The present study highlighted that total volume, white matter volume, and grey matter volume in the cervical spinal segments in ducks could be calculated and revealed by using stereological methods. In the present study, the cervical segments involved in the formation of the cervical enlargement region were also determined.

The present study is presented as an example of morphological and stereological studies to be conducted in anatomy, pathology, and neurobiology.

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