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A stereological study on lumbosacral segments of spinal cord in the geese

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Abstract: In this study, we aimed to determine the number, volume, and lumbosacral enlargement of the lumbosacral segments using stereology methods. In accordance with the study, 10 adult geese (regardless of sex) which were healthy and weighing 3–4 kg was chosen. After the geese had been perfused with 10% formaldehyde, the lumbosacral parts of their spinal cord were revealed by dissection. After each segment of the lumbosacral section had been separated, tissue samples were obtained from these segments. By means of a microtome, sections of 5-μm thickness were taken from the tissue samples. Then, twelve sections were obtained from a lumbosacral segment of each animal at the ratio of 1/250 sampling. Afterwards, all sections of the lumbosacral segments were stained with hematoxylin-eosin and we took the photographs of preparations under a microscope. As a result, all tissues volume fractions and volume values of white and grey matter sections in each segment of the lumbosacral part of the goose were estimated. In addition, the number of lumbosacral segments and lumbosacral enlargement regions was also revealed.

Key words: Goose, lumbosacral segment, medulla spinalis, stereology, volume

1. Introduction

Geese are birds of the Anseriformes order of the Aves class and a member of the Anserinae subfamily of the Anatidae family [1]. In poultry, the spinal cord begins at the foramen magnum in the skull and extends through the vertebral column, including the coccygeal region. [2]. This structure extending to the end of the vertebral canal enters to the phylum terminale which is tapering in the appearance of a thread without forming the cauda equina here**.** As distinct from mammals, spinal cord of poultries is seen in a thin, long and narrow shape. The spinal cord of avians, similar to mammals, is also wrapped with membranes being called under the names of durameter, arachnoidea and pia mater cord. These membranes play a crucial role in the protection of the spinal cord [2–5]. Avians' spinal cord is divided into four segments which are known as cervical part, thoracic, lumbalis and caudal parts. Both in avians and mammals, there is a pair of spinal nerves originating from spinal cords' each segment [6–8]. It is notable that, as seen in mammals, there are also two separate enlargements in poultries named as intumescentia cervical and intumescentia lumbosacral enlargement. It is known that enlargement of cervical segment is bigger in flying birds [3,7,8]. As in avians and mammals, white and gray matter are seen in cross sections obtained from the spinal cord of human being as well [2,9,10]. In the spinal cord, the gray matter is on the inside, while the white matter is on the outside and surrounds the gray matter. The structure, which is located in the middle of the gray matter seen as "H" letter and can also be seen macroanatomically, is the central canal. As distinct from mammals, avians have edge nucleuses which are named as nucleuses marginalis in the gray matter. These nucleuses are observed as masses in avians [5,11]. Grey matter contains ganglion cells, neuritis, afferent myelinated and nonmyelinated fibrils. And also, white matter consists of three parts such as dorsal, lateral and ventral [2,6,10,12]. Stereology is a science that not only examines biological structures in three-dimensional way, but also reveals that three-dimensional objects can be viewed in two-dimensional environments in different branches of science [13,14]. With the use of stereological methods in studies, biased methods used in morphometric studies have been replaced by unbiased methods. Efficiency is as important as objectivity in stereological method. In stereological studies, sequential random sampling forms the basis of studies [15,16]. Different methods are applied to calculate the total volume values of organs or structures and the volume values of their components [17–19]. The Cavalieri's principle is used in stereology in order to calculate total volume values [20,21]. And another calculation method implemented in stereology is point grid [22]. The aim of the

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study is to reveal the total volume value of the lumbosacral segment as well as the volume values of white matter and grey matter in adult geese. It is thought that the reference data obtained may add authenticity and contribution to the studies of knowledge.

2. Materials and methods

2.1. Animals features

In the study, ten healthy and adult geese with no sex difference at an average of 3–4 kg weights were used. The animals were anesthetized by administration of ketamine hydrochloride (50 mg/kg iv) [23]. Ten percentage of formaldehyde solution was used for perfusion with intracardiac method. After perfusion, the geese were soaked in formaldehyde for a good fixation for a week.

2.2. Removal of the spinal cord

In order to dissect the spinal cord of the detected geese, the vertebral arches in the region corresponding to the lumbosacral part of the vertebral column were removed. Thus, the lumbosacral segment was exposed. During this procedure, vertebral column was used as a guide for segment determination [24].

2.3. Sampling method

In stereological studies, it is emphasized that the number of animals must be at least five in order to obtain a 10% ratio of coefficient error, which is accepted close to the truth [17,25]. In the study, it was necessary to consider the total variant square root/whole point number formula in order to be able to conduct coefficient error at section sampling [20]. Tissue specimens were obtained from each segment taken from the lumbosacral part. Having been followed up, tissue specimens were embedded in paraffin. Then, blocked tissues were placed on by a Rotary microtome (Leica LM, 21.35, Nussloch, Germany) as 5-µm thickness sections until the tissue ends, in accordance with the systematic random sampling method from the beginning. Section series were obtained at a pacing ratio of 1/250, with twelve sections from each animal for each segment. After the sections had been deparaffinized, they were stained with hematoxylin eosin and closed with lamellas [24,26]. Since the spinal cord is very small in geese, stereological stepping was not necessary. Under the microscope (Olympus CX31), first surface and then volume calculations were estimated by using point field scale with 4× magnification. After that, SHTEREOM I package program was referred for the calculation [27] (Figure 1). In addition, the Cavalieri's principle was used in the calculations [28]. Total volume values, total volume of grey matter and total volume values of white matter for each segment of fourteen lumbosacral segments of spinal cords in geese were also calculated in this study. In the volume calculations, the number of points were preferred since the numerical ratio of the points can also be included in the evaluation instead of the volume value [29]. When point field scale was being employed, little points were also taken into account for white and grey matter. To prevent unnecessary point account in the whole segment, the result was gained by accounting big points containing quadruple by multiplication number four area of small area in calculation of the whole volume value.

In the calculation of density of volume (ratio of component volume) for each lumbosacral segment, the following formula was employed:

The rate of volume of white matter of each segment = the points of number equivalent to the white matter/the points of number equal to the whole of the segment.

The formula used for volume of each lumbosacral segment in order to calculate the total volume of the lumbosacral segments in volume calculation is that;

The volume of lumbosacral segment = volume rate × reference volume of lumbosacral segment.

Reference volume of lumbosacral segment = section range distance × real surface of the points × the points of number.

3. Results

With the vertebral column guided dissection to the lumbosacral segments of the geese, it was determined that the lumbosacral region consisted of 14 segments.

When the mean volume value of the entire volume of the segment in the lumbosacral segment of the spinal cord in geese was examined, this value was found to be 3.724 mm^3 for the segment LS1. While there was an increasing in the segment LS2, this value decreased to 3.879 mm^3 in the segment LS3; yet, an increase was observed again in the segments LS4, LS5, LS6, LS7, LS8, and LS9 (Figure 2).

On the other hand, the volume value decreased to 4.755 mm^3 in the segment LS10. It was examined that the decrease in the value continued also in the last segment LS14 and was calculated as 1.434 mm^3. Besides, it was determined that LS7 had the highest mean volume with a value of 5.424 mm^3. Furthermore, it was observed that this value which was 3.724 mm^3 in the LS1 increased to 4.915 mm^3 in the LS5. The whole volume value in the LS6 was determined to be 5.092 mm^3. LS6 had the mean volume value of lumbosacral. Then, this volume was followed by the LS7 segment with the highest value of 5.218 mm^3. In addition, this value was determined to be 4.075 mm^3 in the segment LS10. As a result, it was observed that this value decreased more as the number of segments increased. While the whole volume of the lumbosacral part was 2.639 mm^3 in the segment LS12, it was found to be 1.434 mm^3 in the segment LS14. Thus, it was observed that the lowest volume of the lumbosacral segment was the LS14 segment (Figure 3).

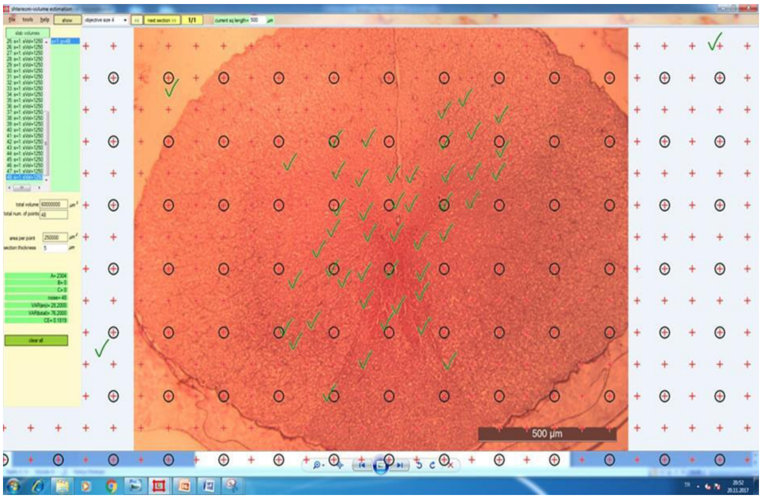

Figure 1. Image analysis with SHTEREOM I program.

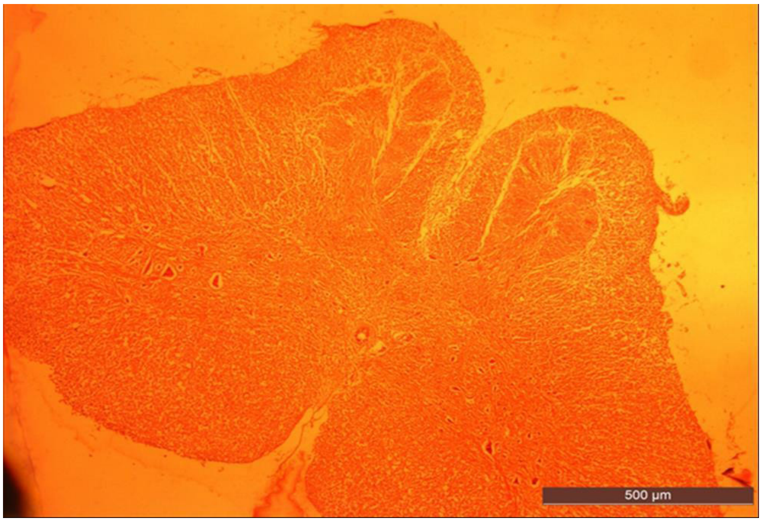

Figure 2. Lumbosacral segment (LS9) in the goose (×4).

Figure 3. Lumbosacral segment (LS11) in the goose (×4).

As a result of the calculation of whole volume values belonging to each of the lumbosacral segments, it was determined that the highest volume values belonged to the segments LS5, LS6, LS7, LS8, and LS9. With the calculations obtained, it was possible to put forward the idea that the enlargement of the lumbosacral region, namely the intumescentia lumbosacralis region, coincides with these segments. The low volume value in the segment LS14, on the other hand, was accepted as an indication of the decrease in the volume value towards the caudal region (Table 1).

When the volume values of white matter in the lumbosacral segment of geese were compared according to the segments, this value was calculated to be 1.974 mm^3 in LS1. This value which increased up to the LS5 segment was identified as 2.550 mm^3 in the LS6. However, this value was found to have increased by 2.729 mm^3 in the LS7. Following this highest value, decreases were observed in the volume value. On the other hand, it was determined that the lowest volume value belonged to the segment LS14. Therefore, it was calculated that the mean volume pertained to the segment LS7 with the value of 2.729 mm^3. It was detected that the volume which increased up to LS7 decreased after then. Additionally, the noticeable decrease was manifested in the LS12 segment with a value of 1.117 mm^3. Nevertheless, it was noticed that the segment LS14 had the lowest volume value. The mean volume of the LS1 was determined to be 1.974 mm^3 (Table 1).

When the grey matter volume values of the lumbosacral segments of geese were evaluated, it was emphasized that this volume value was 1.055 mm^3 in the segment LS1, showed a significant increase in the segment LS5 (Figure 4).

In addition, it was determined that the highest value belonged to LS9 with a value of 1.664 mm^3. While a decrease was observed after following the segment LS10, a significant difference was detected between the LS10 and LS14 segments in terms of calculating volume values. When Table 1 showing volume of grey matter of the spinal cord in the lumbosacral segments was evaluated, it was observed that the highest mean volume of grey matter in the lumbosacral segment belonged to the segment LS9 with a value of 1.664 mm^3. While an increase was detected from the segment LS1 to the segment LS9, a decrease was observed after LS7, though. The lowest ratio of grey matter was calculated to belong to the segment LS14 with a volume of 0.446 mm^3 (Figure 5).

When the white matter/volume of lumbosacral spinal cord were examined, the highest mean value of white matter (WM)/value of lumbosacral spinal cord (LSSC) belonged to the segments LS4 and LS6, and this figure was calculated to be 0.590. The lowest volume value of WM/ LSSC was found to belong to the segment LS12 with a number of 0.440. Following after the segment LS12, the value of WM/ LSSC was found to be 0.556 in LS13 by gradually increasing and 0.572 in LS14 (Table 2).

When Table 2 was analyzed in terms of volume of gray matter (GM)/lumbosacral spinal cord (LSSC), it was calculated that the highest rate belonged to the segment LS1 with the number of 0.369. The lowest value of GM/LSSC was observed to be in the segment LS2 with the number 0.262. On the other hand, numbers among segments were seen to have gradual ups and downs as shown in Table 2.

When the grey matter/white matter table was evaluated, it can be concluded that the highest mean value was found to be in the segment LS1 with the number of 0.714; whereas, the lowest mean value was calculated in the segment LS4 with the number of 0.487 (Table 2).

3.1. Statistical analysis

In calculating the sample size of this study, Power (testing power) was determined by taking at least 80% and type-1 error 5% for each variable. The Shapiro–Wilk ($n < 50$) test was used to check whether the continuous measurements in the study were normally distributed and nonparametric tests were also applied since the measurements were not normally distributed in general. Descriptive statistics in the study was expressed as mean, standard deviation, median, minimum, and maximum. In addition, the Kruskal–Wallis H test was used to compare the measurements according to the groups. Following the Kruskal–Wallis test, the Bonferroni posthoc test was benefited to identify different groups. Separately in groups, Spearman correlation coefficients were calculated to determine the relationships between measurements. Finally, the statistical significance level was taken as 5% in the calculations and the SPSS (IBM SPSS for Windows, v.25) statistical package program was preferred for the calculations. In the statistical comparison of total segment volume between lumbosacral segments in terms of white and gray matter, it was determined that these values differed among themselves. All values are presented in Table 3.

In this study, when the volume values of the whole lumbosacral segments were examined, this value was seen to be the highest in the segment LS7. When the volume values which were obtained in terms of white matter were examined, the highest value was observed in the segment LS7. However, the highest value was detected in the segment LS9 when the grey matter mean volume values were examined. It was underlined that the highest mean lumbosacral segments volume values of the geese belonged to the segment LS7, while the white matter mean volume values were also calculated in the segment LS7. When all the average volume values were examined, the fact that there was a significant increase from LS4 and this value peaked at LS7 provided to bring forward the idea that the region formed by these segments could be the region of

Table 1. Volume values of lumbosacral segment of spinal cord, white matter and grey matter in the geese.

Number of segments	Section	Number of animals										Mean
		G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	
LS1	*WM*	2.796	2.037	2.293	1.410	2.208	2.515	2.510	2.136	0.992	1.850	1.974
	GM	0.985	0.913	1.545	0.702	0.796	2.007	1.907	0.610	0.555	0.531	1.055
	SC	3.601	3.418	4.742	2.608	3.628	5.652	6.116	3.196	2.142	2.137	3.724
LS2	*WM*	1.513	1.883	3.003	3.060	2.328	2.230	2.245	2.028	2.198	1.872	2.236
	GM	1.461	0.946	1.385	0.735	0.788	1.698	1.488	0.513	1.406	0.296	1.118
	SC	4.651	3.403	4.821	5.377	3.793	5.256	5.300	2.943	4.573	4.096	4.421
LS3	*WM*	2.292	1.762	3.672	2.230	2.070	1.882	1.780	1.968	1.062	1.842	2.056
	GM	1.493	1.031	2.160	0.803	1.275	1.540	1.576	0.558	0.662	0.602	1.169
	SC	4.185	3.183	6.980	3.721	4.685	4.220	4.326	2.986	2.260	2.258	3.879
LS4	*WM*	2.776	2.513	3.610	2.350	1.441	2.293	2.265	2.128	1.338	2.071	2.278
	GM	2.042	1.016	2.183	0.757	0.643	1.456	1.350	0.701	0.676	0.625	1.144
	SC	5.437	3.637	7.252	3.858	2.565	4.661	5.073	3.282	2.360	2.338	4.046
LS5	*WM*	3.041	3.157	3.595	2.638	3.350	2.430	2.430	2.001	1.513	2.152	2.630
	GM	2.285	2.482	2.328	0.988	1.358	1.442	1.436	0.686	0.793	0.783	1.458
	SC	5.331	6.252	7.646	4.371	6.565	4.657	5.078	3.300	2.996	2.951	4.915
LS6	*WM*	2.440	3.135	3.130	2.740	2.353	2.376	2.256	2.585	1.906	2.597	2.551
	GM	1.806	2.601	2.261	1.160	1.211	1.496	1.437	0.828	1.117	1.032	1.494
	SC	4.825	6.268	6.788	4.915	5.790	5.325	5.082	3.970	4.021	3.935	5.092
LS7	*WM*	2.448	2.823	2.407	2.948	2.675	3.295	3.278	2.458	2.021	2.327	2.729
	GM	1.423	2.048	1.862	1.265	1.532	2.046	2.085	0.628	1.475	1.398	1.574
	SC	4.777	6.038	4.992	5.348	5.767	7.007	7.337	3.960	4.535	4.480	5.424
LS8	*WM*	1.825	2.796	2.463	3.427	2.848	3.193	3.083	2.558	2.042	2.157	2.639
	GM	1.350	2.137	1.315	1.563	1.212	2.132	2.123	1.262	1.466	1.201	1.576
	SC	3.423	5.821	4.087	6.392	5.135	6.816	7.436	3.717	4.676	4.678	5.218
LS9	*WM*	1.663	1.513	2.726	3.291	2.927	3.183	3.185	3.057	2.356	2.855	2.675
	GM	0.996	1.161	1.403	2.028	1.576	2.081	2.127	1.753	1.828	1.692	1.664
	SC	2.825	2.948	4.202	7.135	5.732	6.773	6.783	5.565	5.433	5.070	5.247
LS10	*WM*	1.358	1.506	3.335	2.701	3.077	1.980	1.890	2.333	1.402	2.638	2.022
	GM	0.928	1.153	2.137	2.027	1.986	1.241	1.335	1.980	1.288	1.278	1.535
	SC	2.387	2.940	6.862	6.213	6.880	4.723	4.566	5.536	3.650	3.788	4.755
LS11	*WM*	0.812	1.720	2.720	2.823	2.843	2.238	2.245	2.350	1.712	2.896	2.235
	GM	0.572	0.977	1.650	1.937	1.623	1.282	1.513	1.545	0.911	0.880	1.289
	SC	1.466	2.755	5.292	5.680	6.156	5.256	4.477	4.596	2.327	2.741	4.075
LS12	*WM*	0.478	1.291	1.482	1.398	1.993	0.835	0.830	0.850	1.196	0.898	1.117
	GM	0.438	0.690	1.201	1.302	1.061	0.548	0.525	0.581	0.577	0.628	0.755
	SC	1.017	2.100	3.173	4.175	3.973	1.672	4.303	1.640	2.176	2.166	2.639
LS13	*WM*	0.435	0.918	0.910	1.401	0.963	0.806	0.806	0.975	0.513	0.918	0.864
	GM	0.386	0.547	0.425	0.742	0.406	0.547	0.533	0.573	0.550	0.381	0.509
	SC	0.908	1.492	1.571	2.651	1.628	1.656	1.651	1.676	1.290	1.123	1.565
LS14	*WM*	0.422	0.872	0.975	1.431	0.918	0.730	0.720	0.805	0.508	0.807	0.795
	GM	0.353	0.522	0.395	0.790	0.382	0.352	0.352	0.393	0.540	0.386	0.446
	SC	0.780	1.481	1.628	2.620	1.667	1.198	1.190	1.206	1.311	1.263	1.434

WM: White Matter, GM: Grey Matter, SC: Spinal Cord (total volume of each segment), G: Goose, LS: Lumbosacral Segment.

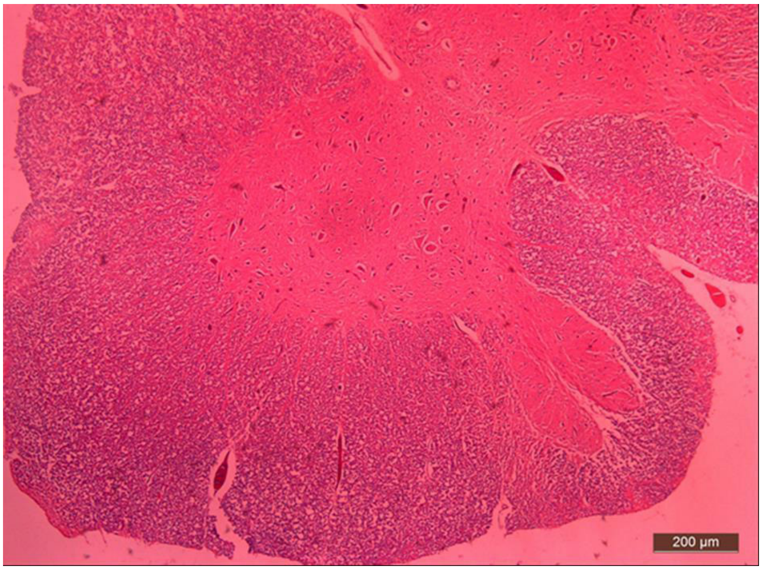

Figure 4. Lumbosacral segment (LS4) in the goose (×4).

Figure 5. Lumbosacral segment in goose (LS14) (×4).

intumescentia lumbosacralis, which is an expansion region. In addition, the fact that the segment range in which the increase in mean volume values started and continued was between segments LS5 and LS9 strengthened the idea that the segments in this range constitute the intumescentia lumbosacralis region. Moreover, in this study, gelatinous structure was found in the dorsal part of the first four segments of intumescentia lumbosacralis. It was thought that this structure might be the corpus gelatinosum filling the sinus rhomboidalis.

4. Discussion

In a study by Begüm et al. [30], while the columna vertebralis was divided into sections, the spinal cord segments were also divided into sections. The spinal segments were exposed by decalcifying the vertebral column. However, in a study on the medulla spinalis, it has been reported that in Leghorn chickens, it is not necessary to segment the spinal cord prior to decalcification and decalcification can be done as a whole [31]. In our study, the geese lumbosacral segments were dissected under the

Table 2. Grey matter volume/white matter volume (GM/WM), white matter volume/spinal cord volume (WM/SC) and grey matter volume/spinal cord volume (GM/SC) ratios of lumbosacral spinal segments in the geese.

Number of segments	Ratio	Number of animals										Mean
		G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	
LS1	*GM/WM*	0.466	0.448	0.673	0.497	0.360	0.798	0.759	0.285	2.575	0.287	0.714
	WM/SC	0.586	0.595	0.483	0.540	0.608	0.444	0.410	0.668	0.463	0.865	0.566
	GM/SC	0.273	0.267	0.375	0.269	0.219	0.355	0.311	0.190	1.192	0.248	0.369
LS2	*GM/WM*	0.661	0.502	0.461	0.240	0.338	0.761	0.662	0.252	0.698	0.692	0.530
	WM/SC	0.475	0.553	0.622	0.569	0.613	0.424	0.423	0.689	0.480	0.457	0.530
	GM/SC	0.314	0.277	0.287	0.136	0.207	0.323	0.280	0.174	0.307	0.316	0.262
LS3	*GM/WM*	0.651	0.585	0.588	0.360	0.615	0.818	0.885	0.283	0.623	0.326	0.573
	WM/SC	0.547	0.553	0.526	0.599	0.441	0.445	0.411	0.659	0.469	0.815	0.546
	GM/SC	0.356	0.323	0.309	0.215	0.272	0.364	0.364	0.186	0.292	0.266	0.294
LS4	*GM/WM*	0.735	0.404	0.604	0.322	0.446	0.634	0.596	0.329	0.505	0.301	0.487
	WM/SC	0.510	0.690	0.497	0.609	0.561	0.491	0.446	0.648	0.566	0.885	0.590
	GM/SC	0.375	0.279	0.301	0.196	0.250	0.312	0.266	0.213	0.286	0.267	0.274
LS5	*GM/WM*	0.751	0.786	0.647	0.374	0.405	0.593	0.590	0.342	0.524	0.363	0.537
	WM/SC	0.570	0.504	0.470	0.603	0.510	0.521	0.478	0.606	0.505	0.729	0.549
	GM/SC	0.428	0.336	0.304	0.226	0.206	0.309	0.282	0.192	0.264	0.265	0.281
LS6	*GM/WM*	0.806	0.791	0.772	0.423	0.514	0.629	0.636	0.320	0.591	0.397	0.587
	WM/SC	0.505	0.500	0.461	0.557	0.406	0.446	0.443	0.651	0.474	0.659	0.590
	GM/SC	0.374	0.414	0.333	0.236	0.209	0.280	0.282	0.208	0.280	0.262	0.287
LS7	*GM/WM*	0.581	0.921	0.773	0.429	0.572	0.620	0.636	0.255	0.728	0.600	0.611
	WM/SC	0.512	0.467	0.482	0.551	0.463	0.470	0.446	0.620	0.445	0.519	0.497
	GM/SC	0.297	0.339	0.372	0.236	0.265	0.291	0.284	0.158	0.325	0.312	0.287
LS8	*GM/WM*	0.739	0.732	0.533	0.456	0.425	0.667	0.688	0.493	0.717	0.549	0.599
	WM/SC	0.533	0.480	0.602	0.536	0.554	0.468	0.414	0.688	0.436	0.461	0.517
	GM/SC	0.394	0.367	0.321	0.244	0.236	0.312	0.285	0.339	0.313	0.256	0.306
LS9	*GM/WM*	0.598	0.767	0.514	0.626	0.538	0.653	0.667	0.573	0.775	0.592	0.630
	WM/SC	0.588	0.513	0.648	0.461	0.510	0.469	0.469	0.549	0.433	0.563	0.520
	GM/SC	0.352	0.393	0.333	0.284	0.274	0.307	0.313	0.315	0.336	0.333	0.324
LS10	*GM/WM*	0.683	0.765	0.640	0.750	0.645	0.626	0.706	0.848	0.918	0.484	0.706
	WM/SC	0.568	0.512	0.486	0.434	0.447	0.419	0.413	0.421	0.384	0.696	0.478
	GM/SC	0.388	0.392	0.311	0.326	0.288	0.262	0.292	0.357	0.352	0.337	0.333
LS11	*GM/WM*	0.704	0.568	0.606	0.686	0.570	0.572	0.673	0.657	0.532	0.303	0.581
	WM/SC	0.553	0.624	0.513	0.497	0.461	0.425	0.501	0.511	0.735	1.056	0.587
	GM/SC	0.390	0.354	0.311	0.342	0.263	0.243	0.337	0.336	0.391	0.378	0.334
LS12	*GM/WM*	0.916	0.534	0.810	0.931	0.532	0.656	0.632	0.678	0.482	0.699	0.687
	WM/SC	0.315	0.614	0.467	0.334	0.501	0.499	0.192	0.518	0.549	0.414	0.440
	GM/SC	0.406	0.328	0.378	0.311	0.267	0.327	0.122	0.354	0.265	0.288	0.304
LS13	*GM/WM*	0.887	0.595	0.467	0.529	0.421	0.678	0.661	0.587	1.072	0.415	0.631
	WM/SC	0.479	0.615	0.579	0.528	0.591	0.486	0.488	0.581	0.397	0.817	0.556
	GM/SC	0.425	0.366	0.270	0.279	0.249	0.330	0.322	0.341	0.426	0.295	0.330
LS14	*GM/WM*	0.836	0.598	0.405	0.552	0.416	0.482	0.488	0.488	1.062	0.478	0.580
	WM/SC	0.541	0.588	0.598	0.546	0.550	0.609	0.605	0.667	0.387	0.638	0.572
	GM/SC	0.452	0.352	0.242	0.301	0.229	0.293	0.295	0.325	0.411	0.294	0.319

WM: White Matter, GM: Grey Matter, SC: Spinal Cord (total volume of each segment), G: Goose, LS: Lumbosacral Segment

Table 3. Comparison of LS measurements by groups (statistical analysis).

	Groups	N	Mean	Std. Dev.	Median	Min.	Max.	*p
LS1	1	10	3.724[a]	1.380	3.510	2.137	6.116	***0.001***
	2	10	2.075[b]	0.541	2.172	0.992	2.796	
	3	10	1.055[c]	0.559	0.855	0.531	2.007	
	4	10	0.715[c]	0.679	0.482	0.285	2.575	
	5	10	0.566[c]	0.133	0.563	0.410	0.865	
	6	10	0.370[c]	0.294	0.271	0.190	1.192	
LS2	1	10	4.421[a]	0.839	4.612	2.943	5.377	***0.001***
	2	10	2.236[b]	0.483	2.214	1.513	3.060	
	3	10	1.072[c]	0.477	1.166	0.296	1.698	
	4	10	0.527[d]	0.196	0.582	0.240	0.761	
	5	10	0.531[d]	0.092	0.517	0.423	0.689	
	6	10	0.262[d]	0.066	0.284	0.136	0.323	
LS3	1	10	3.880[a]	1.384	3.953	2.258	6.980	***0.001***
	2	10	2.056[b]	0.661	1.925	1.062	3.672	
	3	10	1.170[c]	0.528	1.153	0.558	2.160	
	4	10	0.573[cd]	0.200	0.601	0.283	0.885	
	5	10	0.547[cd]	0.122	0.537	0.411	0.815	
	6	10	0.295[d]	0.061	0.301	0.186	0.364	
LS4	1	10	4.046[a]	1.577	3.748	2.338	7.252	***0.001***
	2	10	2.279[b]	0.644	2.279	1.338	3.610	
	3	10	1.145[c]	0.589	0.887	0.625	2.183	
	4	10	0.488[cd]	0.151	0.476	0.301	0.735	
	5	10	0.590[cd]	0.128	0.564	0.446	0.885	
	6	10	0.274[d]	0.051	0.273	0.196	0.375	
LS5	1	10	4.915[a]	1.587	4.868	2.951	7.646	***0.001***
	2	10	2.631[b]	0.654	2.534	1.513	3.595	
	3	10	1.458[c]	0.684	1.397	0.686	2.482	
	4	10	0.538[d]	0.163	0.557	0.342	0.786	
	5	10	0.550[d]	0.079	0.516	0.470	0.729	
	6	10	0.281[d]	0.069	0.273	0.192	0.428	
LS6	1	10	5.092[a]	0.980	4.999	3.935	6.788	***0.001***
	2	10	2.552[b]	0.380	2.513	1.906	3.135	
	3	10	1.495[c]	0.568	1.324	0.828	2.601	
	4	10	0.588[d]	0.172	0.610	0.320	0.806	
	5	10	0.510[d]	0.087	0.487	0.406	0.659	
	6	10	0.288[d]	0.068	0.280	0.208	0.414	
LS7	1	10	5.424[a]	1.110	5.170	3.960	7.337	***0.001***
	2	10	2.668[b]	0.417	2.567	2.021	3.295	
	3	10	1.576[c]	0.453	1.504	0.628	2.085	
	4	10	0.611[d]	0.182	0.610	0.255	0.921	
	5	10	0.498[d]	0.055	0.476	0.445	0.620	
	6	10	0.288[d]	0.059	0.294	0.158	0.372	

Table 3. (Continued).

LS8	1	10	5.218^{a}	1.357	4.906	3.423	7.436	***0.001***
	2	10	2.639^{b}	0.525	2.677	1.825	3.427	
	3	10	1.576^{c}	0.398	1.408	1.201	2.137	
	4	10	0.600^{d}	0.121	0.608	0.425	0.739	
	5	10	0.517^{d}	0.083	0.506	0.414	0.688	
	6	10	0.307^{d}	0.052	0.313	0.236	0.394	
LS9	1	10	5.247^{a}	1.524	5.499	2.825	7.135	***0.001***
	2	10	2.676^{b}	0.634	2.891	1.513	3.291	
	3	10	1.665^{c}	0.385	1.723	0.996	2.127	
	4	10	0.630^{d}	0.088	0.612	0.514	0.775	
	5	10	0.520^{d}	0.067	0.512	0.433	0.648	
	6	10	0.324^{d}	0.034	0.324	0.274	0.393	
LS10	1	10	4.755^{a}	1.591	4.645	2.387	6.880	***0.001***
	2	10	2.222^{b}	0.706	2.157	1.358	3.335	
	3	10	1.535^{c}	0.444	1.312	0.928	2.137	
	4	10	0.707^{d}	0.122	0.695	0.484	0.918	
	5	10	0.478^{d}	0.094	0.441	0.384	0.696	
	6	10	0.331^{d}	0.043	0.332	0.262	0.392	
LS11	1	10	4.075^{a}	1.619	4.537	1.466	6.156	***0.001***
	2	10	2.236^{b}	0.664	2.298	0.812	2.896	
	3	10	1.289^{c}	0.435	1.398	0.572	1.937	
	4	10	0.587^{d}	0.116	0.589	0.303	0.704	
	5	10	0.588^{d}	0.187	0.512	0.425	1.056	
	6	10	0.335^{d}	0.050	0.340	0.243	0.391	
LS12	1	10	2.640^{a}	1.178	2.171	1.017	4.303	***0.001***
	2	10	1.125^{b}	0.435	1.047	0.478	1.993	
	3	10	0.755^{bc}	0.311	0.605	0.438	1.302	
	4	10	0.687^{bc}	0.157	0.667	0.482	0.931	
	5	10	0.440^{c}	0.127	0.483	0.192	0.614	
	6	10	0.305^{c}	0.079	0.319	0.122	0.406	
LS13	1	10	1.565^{a}	0.462	1.600	0.908	2.651	***0.001***
	2	10	0.864^{b}	0.265	0.914	0.435	1.401	
	3	10	0.509^{cd}	0.112	0.540	0.381	0.742	
	4	10	0.631^{c}	0.209	0.591	0.415	1.072	
	5	10	0.556^{cd}	0.113	0.554	0.397	0.817	
	6	10	0.330^{d}	0.061	0.326	0.249	0.426	
LS14	1	10	1.434^{a}	0.488	1.287	0.780	2.620	***0.001***
	2	10	0.819^{b}	0.276	0.806	0.422	1.431	
	3	10	0.447^{c}	0.138	0.390	0.352	0.790	
	4	10	0.581^{c}	0.209	0.488	0.405	1.062	
	5	10	0.573^{c}	0.077	0.593	0.387	0.667	
	6	10	0.319^{d}	0.070	0.298	0.229	0.452	

** Significance levels according to Kruskal–Wallis test results.*
a,b,c: Shows the difference between groups according to the Bonferroni posthoc test.

guidance of the vertebral column while the spinal cord was attached to the vertebral column after the vertebral arc was removed. Can et al. [32] reported that the dorsal vertebra of the synsacrum is formed by the fusion of all lumbar, sacral and the first five tail vertebrae. Besides, in a study conducted by Bolat [31], fourteen lumbosacral vertebrae were detected in Leghorn female and male chickens. As distinct from the study by Can et al. [32], it was determined that lumbosacral vertebra was formed by the union of the lumbar and sacral vertebra in the teal. In addition, fourteen lumbosacral vertebrae were identified in this study. In this respect, current study shows similarities with the study conducted by Bolat [31] on Leghorn chickens. When the present literature is reviewed, the number of lumbar vertebra is stated to be four in duck, goose, pigeon and chicken [2,33]. Moreover, 16 lumbosacral segments in ducks, 14 in chickens and 13 in pigeons were mentioned in a study carried on chicken, duck and pigeon [9]. In a different study on the teal, Can et al. [32] stated the number of lumbar vertebra as four. However, in another study, 14 lumbosacral segments were mentioned in chicken [34]. Chiasson [35] determined 21 lumbosacral segments in his study on pigeons. In another study on spinal cord in poultry, it was found that the spinal cord contains 14 lumbosacral segments in Leghorn chickens [31]. In this study, an overlap was also detected in terms of the number of lumbosacral segments with the research done by Bolat [31]. This study carried on geese shows differences from other studies in terms of the number of lumbosacral segments. In a study on lumbosacral segments in horses by Selçuk [36], volume value calculations were determined by the Cavalieri principle and an optical dissector was preferred. In another study on the spinal cord of Leghorn chickens, an optic dissector was also preferred [31]. In this study, volume value calculations were made with the Cavalieri principle in addition to a physical dissector that can clearly reflect realistic results. Although the study is similar to the studies of Bolat [31] and Selçuk [36] in terms of using the Cavalieri principle in volume value calculations, it also differs from these studies by the preference of the physical dissector. Hazıroğlu et al. [9] emphasized that intumescentia lumbalis in chickens were formed by LS1–LS8 segments, in pigeons LS1–LS12 segments and in ducks LS1–LS11 segments. Based on a different volume study performed in Leghorn chickens, it was concluded that the lumbosacral enlargement was the widest part of the spinal cord sections [31]. On the other hand, it was stated the lumbosacral enlargement to be the widest part in a different study made from the transversal sections of the spinal cord [37]. Furthermore, it was found out that lumbosacral enlargement is located between LS1–LS6 in Çakmak's and Karadağ's study on the lumbosacral segments of quails [38]. Apart from Çakmak's and Karadağ's [37] study, lumbosacral enlargement was found to be between LS4 and LS9 in this study. In a study on the spinal cord in poultry, it was reported that central canal is completely located in the corpus gelatinosum in the segments LS5–LS11 in ducks, segments LS6–LS10 in chickens, and segments LS2–LS9 in pigeons [8]. In a study conducted in Leghorn chickens, it was determined that the sinus rhomboidalis, which is located in the dorsal part of the last thoracal segment and the first three segments of the intumescentia lumbalis, was filled by the corpus gelatinosum [31]. However, in this study, it was revealed that the corpus gelatinosum filled the sinus rhomboidalis in the lumbosacral enlargement section. Rahmanifar et al. [39] reported that the amount of grey matter in thoracic part was the least in adult ostriches. In a study on the cervical segment in quails, the volume of gray matter was found to be less than the volume of white matter [24]. In this study, similarly, when volume values of the gray and white matter of the lumbosacral segment were examined, it was revealed that volume of the gray matter was lower than the volume of white matter. In volume calculation studies done by using the Cavalieri principle, it was emphasized that the coefficient of error should be 10% or less as an important parameter [20,40]. In our study, the coefficient of error was determined separately for the all segment and volume values of white and grey matter, and this reported value was found to be reliable for the study, as well. As a result, it was determined that the whole segment, gray and white matter parts between spinal cord lumbosacral segments show differences in terms of the volumes in adult geese. It has been determined that the white and grey matter parts of the segments of the lumbosacral section and volume values of all segment in geese from poultry can be evaluated separately and methods of stereology are in need so as to reveal the closest value from the obtained values. In addition, with the help of this study, it was revealed that parameters such as volume and cell count that can be done for all segments of the spinal cord in geese may be insufficient and separate sampling strategy seems to be necessary for each segment. Thus, it was detected in this study that the physical dissector could be preferred in the sampling strategy. We believe that stereological methods can be used in all studies on the nervous system in poultry so that new studies that have reference value might be supported by this study and this study might lead other studies in literature. Moreover, the fact that studies in the field should not be limited has also been revealed with this study.

Acknowledgments/conflict of interest

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