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A study on morphological and morphometrical parameters on the skull of the Konya Merino Sheep

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ABSTRACT

Objective: In this study, it was aimed to determine the craniometric measurements of the skull of the Konya Merino breed and to reveal the differences between it and other sheep breeds.

Material-Method: For this study, ten Konya merino heads were used and craniometric measurements were made from 46 points on the skull.

Result: In the study, the skull length of the Konya merino was 274.03±5.23, the frontal width (ectorbitale – ectorbitale) which is the widest region of the skull was 123.47 ± 2.60, zygomatic width (the distance between two zygomatic arches) was 110.30±1.96 and the distance between the foramen supraorbitales was 49.06 ± 2.38.. It was determined that both the fronto-nasal and palato-maxillar sutures resembled the letter "V" in Konya merino, while the pariato-frontal suture was in the form of a straight line. When the correlation between index values was examined, it was seen that there was a statistically significant strong positive correlation between I1 (Nasal index) and I2 (Facial Index), but the relationship between other examined features was insignificant. When the statistically significant correlation values of the skull measurements were examined, it was seen that there was a strong negative or positive correlation between the features. While the highest positive correlation was between L5 (premolare – prosthion) and L39 (greatest palatal breadth (measured across the outer borders of the alveoli) features (0.943), the highest negative correlation was L33 (greatest neurocranium breadth-greatest breadth of the alveoli). Braincase (euryon - euryon) and L46 (supraorbital foramina distance) features were found to be (-0.908).

Conclusion: As a result, in this study, it is thought that the difference in the craniometric values of Konya merino, which is accepted as the native breed of Turkey, with other sheep breeds, depending on the skull morphology, may be caused by the breed of sheep.

Keywords: *Konya merino sheep, Morphology, Craniometrics, Skull*

INTRODUCTION

The skull is a structure consisting of a series of fused flat and mostly double bones. This structure includes the brain as well as the sense organs such as vision, smell, balance and taste. The upper respiratory and digestive tracts are also located in here. It is divided into two parts as neurocranium and viscerocranium. Neurocranium consists of cavum cranii and viscerocranium consists of bones that make up the facial skeleton (König and Liebich, 2020).

Taking craniometric measurements depending on skull morphology is a preferred method in

zooarchaeological studies, osteological evaluations, to reveal shape differences due to internal and external factors and differences between sexes (Cakir et al., 2012).

Konya Merino, also called Central Anatolian Merino, is one of Turkey's domestic sheep breeds, obtained by crossing the German fleece meat merino and Akkaraman sheep. It is a breed developed by crossbreeding studies carried out in Konya stud farm since 1950. Konya Merino breed carries an average of 80% German fleece meat merino and 20% Akkaraman genotype. The sheep, which has a large, deep, wide and long body structure, has a medium head length and width, and thick lips. The face and legs of the sheep are bare, the fleece is thin and uniform, with a white fleece. The tail is lean, thin and long. Sheep and rams are hornless (Akcapinar, 1994).

To this date, some studies on Konya merino (Ozudogru et al., 2019; 2021) and craniometric measurements have been carried out in Xisqueta sheep (Parés-Casanova et al., 2010), Tuj and Morkaraman sheep (Ozcan et al., 2010), Mehraban sheep (Karimi et al., 2011), Iranian domestic sheep (Monfared, 2013), Barbados Black Belly sheep (Mohamed et al., 2016), Sharri sheep (Jashari et al., 2022), Hasmer and Hasak sheep (Can et al., 2022) and South Karaman sheep (Ozudogru et al., 2022) no craniometric studies were found in Konya merino. This study is especially important because it is the first study on the head structure of Turkey's native sheep breed, which is common in the Central Anatolian region.

MATERIALS and METHODS

Supply of Materials

In the study, 10 Konya merino skulls with a weight varying between 44-79 kg obtained from Bahri Dağdaş International Agricultural Research Institute were used.

After the animals were duly slaughtered, the skulls were subjected to maceration. Measurements were made using Mitutoyo digital caliper from 46 points on the Konya Merino skull. The anatomical terms used were based on Nomina Anatomica Veterinaria (NAV, 2017).

Statistical analysis: The mean values, standard deviations, coefficient of variations and craniofacial indices were calculated with SPSS (version 22). Independent samples *t* test was used for *p* values. The values determined are indicated in Tables 1-4.

This study was approved by the Experimental Animals Ethics Committee of Atatürk University (Ethical number: 23.10.2015, 8/148).

Measuring points on the skull;

Akrokranion (A): the most aboral point on the vertex of the cranium in the median plane,

Basion (B): the orobasal border of the foramen magnum in the median plane,

Bregma (Br): the median point of the parieto-frontal suture,

Ectorbitale (Ect): the most lateral point of the frontal bone on the occipital side of the orbit,

Entorbitale (Ent): the naso-medial indentation of the orbit that corresponds with the inner angle of the eye in the living animal,

Euryon (Eu): the most lateral point of the braincase,

Infraorbitale (If): the (dorso) aboral point of the foramen infraorbitale,

Nasion (N): the median point of the naso-frontal suture,

Nasointermaxillare (Ni): the most aboral point of the premaxilla on the facial surface,

Opisthion (O): the nuchodorsal border of the foramen magnum in the median plane,

Otion (Ot): the most lateral point of the mastoid region,

Prosthion (P): the median point of the line joining the most oral points of the premaxillae,

Postdentale (Pd): the median point of the line joining the aboral points of the alveoli of the hindmost cheekteeth,

Premolare (Pm): the median point of the line joining the oral points of the alveoli of the foremost cheekteeth,

Palatinoorale (Po): the median point of the palatine-maxillary suture,

Rhinion (Rh): the median point of the line joining the most oral points of the nasals, Supraorbitale (Sp): the median point of the line joining the aboral borders of the supraorbital foramina (Von den Drisch, 1976).

The following measurements by using definitions of measuring points (Onar and Pazvant, 2001; Ozcan et al., 2010; Dalga et al., 2018; Ozkan et al., 2019; Gundemir et al., 2020) on the cranium were made:

L1. profile length (akrokranion - prosthion),

L2. condylobasal length (aboral border of occipital condyles - prosthion),

L3. basal length (basion - prosthion),

L4. short skull length (basion premolare),

L5. premolare - prosthion,

L6. neurocranium length (basion - nasion),

L7. viscerocranium length (nasion prosthion),

L8. median frontal length (akrokranion - nasion),

L9. akrokranion - bregma,

L10. frontal length (bregma - nasion),

L11. upper neurocranium length: Akrokranion - supraorbitale,

L12. facial length (supraorbitale - prosthion),

L13. akrokranion-infraorbitale of one side,

L14. greatest length of the lacrimal (most lateral point of the lacrimal - the most oral point of the lacrimo-maxillary suture,

L15. greatest length of the nasals (nasion-rhinion),

L16. short lateral facial length (entorbitale - prosthion),

L17. from the aboral border of one occipital condyle to the infraorbitale of the same side,

L18. dental length (postdentale - prosthion),

L19. oral palatal length (palatinoorale - prosthion),

L20. lateral length of the premaxilla (nasointermaxillare - prosthion),

L21. length of the cheektooth row (measured along the alveoli),

L22. length of the molar row (measured along the alveoli on the buccal side),

L23. length of the premolar row (measured along the alveoli on the buccal side),

L24. zygomatic width (the distance between two zygomatic arches)**,**

L25. greatest inner length of the orbit (ectorbitale - entorbitale),

L26. greatest inner height of the orbit (measured in the same way as measurement),

L27. greatest mastoid breadth (otion - otion),

L28. greatest breadth of the occipital condyles,

L29. greatest breadth at the bases of the paraoccipital processes,

L30. greatest breadth of the foramen magnum,

L31. eight of the foramen magnum (basion - opisthion),

L32. least breadth of parietal: Least breadth between the temporal lines,

L33. greatest neurocranium breadth-greatest breadth of the braincase (euryon - euryon),

L34. least breadth between the orbits (entorbitale - entorbitale),

L35. greatest breadth across the orbit-greatest frontal breadth-greatest breadth of the skull (ectorbitale - ectorbitale),

L36. facial breadth (breadth across the facial tuberosities),

L37. greatest breadth across the nasals,

L38. greatest breadth across the premaxillae,

L39. greatest palatal breadth (measured across the outer borders of the alveoli).

L40. the distance from infraorbital foramen to facial tuberosity**,**

L41. the distance from facial tuberosity to the root of the alveolar tooth,

L42. distance between first premolar teeth,

L43. distance between first molar teeth,

L44. distance between the last molar teeth,

L45. distance from orbital arcus,

L46. supraorbital foramina distance.

Craniofacial indices (Ozcan et al., 2010; Gundemir et al., 2020)**:**

I1. Nasal index: greatest breadth across the nasals x 100/ greatest length of the nasals

I2. Facial index: zygomatic width x 100/viscerocranial length

I3. Neurocranium index: maximum width of the neurocranium x 100/neurocranium length

I4. Basal index: maximum width of neurocranium x100/basal length

I5. Skull index: zygomatic width x 100/skull length

I6. Orbital index: Greatest inner height of the orbit x100/ Greatest inner length of the orbit

I7. Foramen Magnum index: The height of the foramen magnum× 100/the width of the foramen magnum.

RESULTS

In the study, 46 morphometric measurements of Konya Merino were made. The reference points for these measurements are given in Figure 1-6, the morphometric values obtained are presented in Table 1, and the calculated index values are presented in Table 2.

As seen in Table 1, the skull length of the Konya merino was 274.03±5.23, the frontal width (ectorbitale – ectorbitale) which is the widest part of the skull was 123.47 ± 2.60, the distance between the

foramen supraorbitales was 49.06±2.38 and the distance between arcus zygomaticus was 110.30 ± 1.96.

Table 1. The mean and standard deviations values of Merino sheep.

Length	Mean ± Std Deviation	Length	Mean ± Std Deviation
L1	274.03 ± 5.230	L24	110.30 ± 1.957
L2	255.76 ± 3.030	L25	43.69 ± 1.756
L3	237.27 ± 2.336	L26	38.34 ± 1.950
L4	170.15 ± 3.310	L27	74.62 ± 2.252
L5	70.14 ± 2.429	L28	48.89 ± 0.957
L6	124.82 ± 3.958	L29	70.33 ± 2.218
L7	149.04 ± 5.136	L30	22.87 ± 1.807
L8	133.03 ± 1.910	L31	20.03 ± 0.728
L9	52.46 ± 3.200	L32	50.62 ± 0.994
L10	90.58 ± 2.839	L33	68.89 ± 1.215
L11	105.06 ± 2.499	L34	88.59 ± 0.948
L12	134.38 ± 4.498	L35	123.47 ± 2.605
L13	200.07 ± 4.461	L36	89.99 ± 2.015
L14	45.73 ± 2.909	L37	37.53 ± 1.187
L15	98.41 ± 4.878	L38	46.53 ± 1.818
L16	156.08 ± 4.827	L39	79.86 ± 2.488
L17	180.97 ± 1.362	L40	30.35 ± 1.940
L18	136.01 ± 2.799	L41	14.34 ± 0.947
L19	110.49 ± 2.972	L42	31.77 ± 1.176
L20	88.08 ± 1.929	L43	42.97 ± 1.069
L21	75.43 ± 4.116	L44	50.29 ± 2.087
L22	51.98 ± 3.351	L45	104.42 ± 1.996
L23	24.78 ± 2.057	L46	49.06 ± 2.384

Table 2. The mean and standard deviation values of craniofacial indices of Merino sheep.

Craniofacial index	Mean ± Std Deviation
I1 Nasal index	74.08 ± 2.804
I2 Facial index	38.22 ± 2.263
I3 Neurocranium index	55.25 ± 2.354
I4 Basal index	29.04 ± 0.560
I5 Skull index	40.26 ± 0.992
I6 Ortibal Index	114.16 ± 6.873
I7 For. Mag. Index	88.03 ± 7.135

When Table 3, which indicates the correlation between index values, is examined, it is seen that there is a statistically significant strong positive correlation between I1 (Nasal index) and I2 (Facial Index), but the relationship between other examined features is insignificant. Although the correlation between I3 and I4 index values was high (r=0.721), the correlation was found to be statistically insignificant (P=0.058). A similar situation was seen in I1 and I7 features (P=0.094).

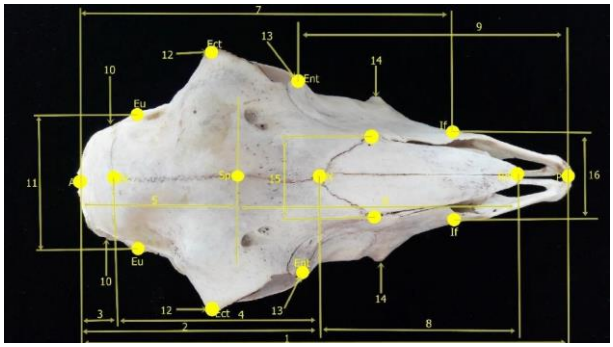

Figure 1. Measurements of the skull of the Konya merino sheep (dorsal view).

1. profile length (akrokranion - prosthion), **2.** median frontal length (akrokranion - nasion), **3.** akrokranion-bregma, **4.** frontal length (bregma - nasion), **5.** upper neurocranium length (Akrokranion – supraorbitale), **6.** facial length (supraorbitale - prosthion),**7.** akrokranion-infraorbitale of one side, **8.** greatest length of the nasals (nasion-rhinion), **9.** short lateral facial length (entorbitale - prosthion), **10.** least breadth of parietal: Least breadth between the temporal lines, **11.** greatest neurocranium breadth-Greatest breadth of the braincase (euryon - euryon),**12.** greatest breadth across the orbit-greatest frontal breadth-greatest breadth of skull (ectorbitale - ctorbitale),**13.** least breadth between the orbits (entorbitale - entorbitale),**14.** facial breadth (breadth across the facial tuberosities),**15.** greatest breadth across the nasals,**16.** greatest breadth across the premaxillae.

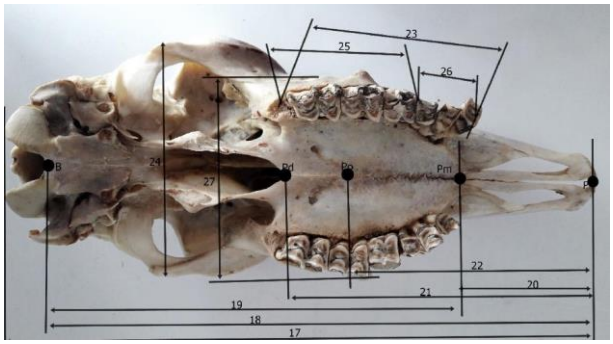

Figure 2. Measurements of the skull of the Konya merino sheep (ventral view).

17. condylobasal length (aboral border of occipital condyles - prosthion), **18.** basal length basion - prosthion), **19.** short skull length (basion premolare), **20.** premolare-prosthion, **21.** dental length (postdentale - prosthion), **22.** oral palatal length (palatinoorale - prosthion), **23.** Length of the cheektooth row (measured along alveoli), **24.** zygomatic width (the distance between two zygomatic arches), **25.** Length of the molar row (measured along the alveoli on the buccal side), **26.** Length of the premolar row (measured along the alveoli on the buccal side), **27.** greatest palatal breadth (measured across the outer borders of the alveoli).

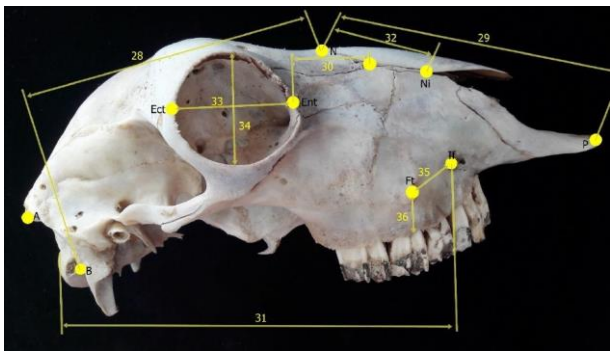

Figure 3. Measurements of the skull of the Konya merino sheep (lateral view).

28. neurocranium length (basion - nasion), **29.** viscerocranium length (nasion-prosthion), **30.** greatest length of the lacrimal (most lateral point of the lacrimal - the most oral point of the lacrimo-maxillary suture, **31.** from the aboral border of one occipital condyle to the infraorbitale of the same side, **32.** lateral length of the premaxilla (nasointermaxillare - prosthion), **33.** greatest inner length of the orbit (ectorbitale - entorbitale),**34.** greatest inner height of the orbit (measured in the same way as measurement), 35**.** the distance from infraorbital foramen to facial tuberosity**, 36.** the distance from facial tuberosity to root of alveolar tooth,

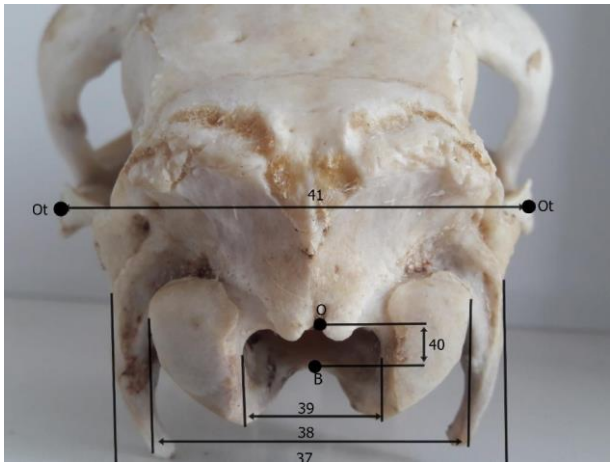

Figure 4. Measurements of the skull of the Konya merino sheep (occipital view)

37. greatest breadth of the bases of the paraoccipital processes,

38. greatest breadth of the occipital condyles,

39. greatest breadth of the foramen magnum,

40. height of the foramen magnum (basion - opisthion),

41. greatest mastoid breadth (otion - otion).

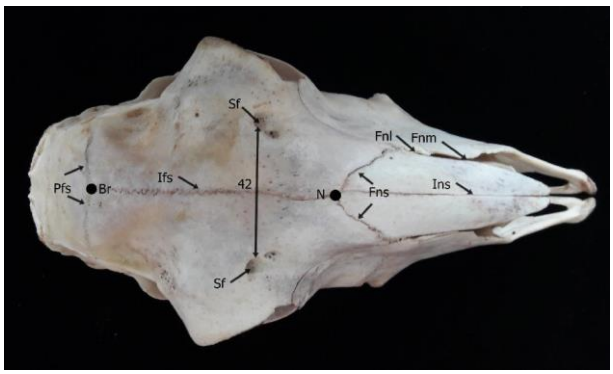

Figure 5. Morphological feature of the skull of Konya merino (frontal surface).

42. Supraorbital foramina distance; **Nfs.** Naso-frontal suture like "U" shape; **Pfs.** Parieto-frontal suture like "V" shape; **Ins.** Internasal suture (caudal quarter part is serrated); **Sf.** supraorbital foramen; **Fnm.** fissura nasomaxillaris; **Fnl.** fissura nasolacrimalis; **Ifs.** Interfrontal sutura.

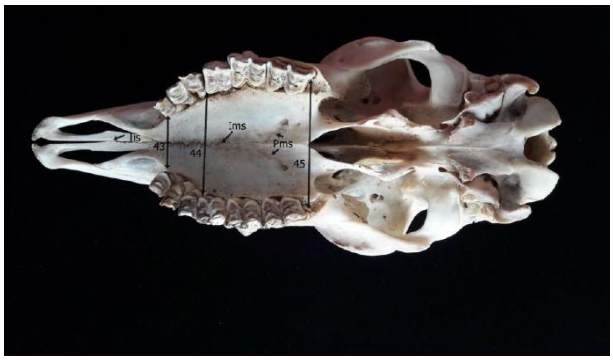

Figure 6. Morphological feature of the skull of Konya merino (basal surface).

43. distance between first premolar teeth, **44.** distance between first molar teeth, **45.** Distance between the last molar teeth, **Pms.** Palato-maxillar sutura ("V" shape of the palatine bone with maxilla's palatine processes); **Ims**. Intermaxillar sutura; **Iis.** Interincisiv sutura.

Table 3. Correlation of craniofacial index.

	I1	I2	I3	I4	I5	I6
I2	0.796*					
I3	0.177	0.396				
I4	0.248	0.231	0.721			
I5	0.348	0.403	-0.236	-0.250		
I6	-0.123	-0.085	0.138	-0.278	0.461	
I7	0.678	0.203	-0.439	0.004	0.164	-0.304

*: p<0.05; **: p<0.01

Statistically significant correlation values of skull measurements are shown in Table 4. When Table 4 is examined, it is seen that there is a strong negative or positive correlation between the characteristics. While the highest positive correlation was between L5 and L39 features (0.943), the highest negative correlation was between L33 and L46 features (-0.908).

Table 4. The correlation values of skull Merino sheep.

	L1	L2	L3	L4	L5	L6	L7	L8	L10	L12	L13	L14	L15	L16
L5		0.876**												
L8						0.779*								
L9					0.809*									
L10					0.872*									
L11								0.797*						
L12			0.786*				0.775*							
L13	0.900**	0.867*												
L14	-0.758*	0.790*									0.856*			
L15							0.936**			0.857*				
L16							0.868*						0.831*	
L18		0.845*			0.836*					0.845*		0.862*		0.824*
L25									0.828*					
L26				-0.783*										
L28							0.776*							
L33				0.787*										
L34						0.773*								
L36										0.886**		0.759*		
L38							0.917**			0.849*			0.933**	0.935**
L39		0.788*			0.943**				0.872**					
L42							-0.810*							-0.796*
L43	-0.808*	0.882**									0.869*	0.845*		
L44									-0.858*					

*: p<0.05; **: p<0.01

DISCUSSION

In the study, the skull length was determined as 274.03 ± 5.23 in Konya merino. This value is 209±4.77 in Iranian domestic sheep (Monfared 2013), 200.6±0.6 in Mehraban sheep (Karimi et al., 2011), 246.5±2.16 in Barbados Black Belly sheep (Mohamed et al., 2016), 198.08±7.69 in Tuj sheep and 204.49±9.71 in Morkaraman sheep (Ozcan et al. 2010), 241.20±25.17 in Hemsin sheep (Dalga et al., 2018), Suffolk Down Sheep (Barra et al., 2020) 238.3±2.07, Kosova in Barkhoka sheep (Gundemir et al., 2020) 245.25±10.24, Zell sheep (Marzban Abbasabadi et al., 2020) 196.73±0.60 in Yankasa sheep (Shehu et al., 2019) 325±0.99 in Awassi sheep (Yilmaz and Demircioğlu, 2020) was reported to be 241.30±14.01, in Xisqueta sheep (Parés-Casanova et al., 2010) 265.51± 22.24 and in Sharri sheep (Jashari et al., 2022) 247.47 ± 13.12. According to these reported values, it was observed that the skull length of the Konya merino was longer than all of the other reported species except for the skull length of the Yankasa sheep.

The skull index value in Morkaraman sheep (Ozcan et al. 2010) is 51.36±0.69, Tuj sheep (Ozcan et al. 2010) 50.42±0.78, Mehraban sheep (Karimi et al., 2011) 53.57±3.26, Awassi sheep (Yılmaz and Demircioğlu, 2020) 47.77±3.23, Xisqueta sheep (Parés-Casanova et al., 2010) 44.69± 4.29, Barkhoka sheep of Kosova (Gundemir et al., 2020) 41.69±1.74, Saanen goat (Wang et al., 2021), hasmer sheep (Can et al., 2022) 46.36, South Karaman sheep (Ozudogru et al., 2022) 42.16 ± 1.06 was measured as 53.45±1.55. This value was measured as 40.26 ± 0.992 in Konya merino sheep.

In dogs (Onar and Pazvant, 2001), camels (Al-Sagair and Al-Mougy, 2002) and Kagani goats (Sarma, 2006), the distance between the two arcus zygomaticus has been reported as the widest region of the skull. Yılmaz and Demircioğlu (2020) and Ozcan et al. (2010) stated that the widest region of the skull in sheep is the frontal width (ectorbitale – ectorbitale) due to morphological differences. Accordingly, they reported that this length was 102.98±2.52 mm in Morkaraman sheep (Ozcan et al. 2010), 101.66±1.69 mm in Tuj sheep (Ozcan et al. 2010), and 115.07±7.74 mm in Awassi sheep (Yilmaz and Demircioğlu, 2020). In this study, the distance between two arcus zygomaticus in Konya merino was 110.30 ± 1.96 and the frontal width (ectorbitale – ectorbitale) was measured as 123.47 ± 2.60. According to these values, it was determined that

the widest region of the skull was the frontal width (ectorbitale – ectorbitale) in Konya merino.

From a clinical point of view, since the nervus infraorbitalis innervates the lateral and upper parts of the nose, upper lip and facial skin, it is important to determine the location of the foramen infraorbitale in the blockade of this nerve (Getty, 1975). To locate the foramen infraorbitale, it is necessary to know the distance from the infraorbital foramen to facial tuberosity. This distance has been reported as 18.7 ± 0.09 in Iranian domestic sheep (Monfared, 2013), 31.6±0.70 in Barbados Black Belly sheep (Mohamed et al., 2016) and 12.82±0.18 in female Zell sheep skull (Marzban Abbasabadi et al., 2020). In Konya merino, this distance was measured as 30.35±1.94. Since the orbital region consists of a complex bone structure, it plays a fundamental role in the evaluation and recognition of the craniofacial complex.

Parés-Casanova et al. (2010), in their study on the biometric appearance of the skull in Spanish Xisqueta sheep, reported that the orbital index value was 109.77±10.23. The mentioned orbital index was measured as 112.27±3.50 in the Awassi sheep (Yilmaz and Demircioglu, 2020) and 93.46± 3.48 in the Barkhoka sheep of Kosova (Gundemir et al., 2020). In the study, it was determined that the value measured as 114.16 ± 6.87 in Konya merino was greater than all of the mentioned sheep species.

Although it was stated that the fronto-nasal sutura was in the form of the letter "V" in Sharri sheep (Jashari et al., 2022), in Bardhoka sheep of Kosovo (Gundemir et al., 2020), Kagani goat (Sarma, 2006), and Hemsin sheep (Dalga et al., 2018) it has been reported to be in the shape of the letter “U”. In the study, it was determined that the fronto-nasal sutura resembles the letter "V" in Konya merino.

It is reported that the palato-maxillary sutura between the lamina horizantali of the os palatine and the processus palatinus of the os maxilla is in the form of the letter "U" in hellon sheep (Karimi et al., 2011), and in the shape of the letter "V" in Bardhoka sheep of Kosovo (Gundemir et al., 2020) has been done. In the study, it was determined that the palato-maxillar sutura in Konya merino resembles the letter "V" as in Bardhoka sheep. In addition, in the study, it was determined that the pariato-frontal sutura was in the form of a straight line, and this finding is consistent with the reports of Sharri sheep (Jashari et al. 2022) that the sutura can be in the form of a straight line or the letter "V".

When the correlation between the index values of Konya merino is examined in the study, it is seen that there is a statistically significant strong positive correlation between I1 (Nasal index) and I2 (Facial Index), while the relationship between other examined features is insignificant. Although the correlation between I3 (Neurocranium index) and I4 (Basal index) index values was high (r=0.721), the correlation was found to be statistically insignificant (p=0.058).

In their study on Hemsin sheep skull index values, Dalga et al. (2018) found that there was a statistically significant and strong positive correlation between the Neurocranium index and Basal index.

According to the statistical values of Konya Merino skull measurements, it is seen that there is a strong negative or positive correlation between the features. The highest negative correlation was found between L33 (greatest neurocranium breadth-greatest breadth of the braincase (euryon - euryon) and L46 (supraorbital foramina distance) features (-0.908).

There is a positive strong correlation between the basal length and the short skull length of the hemsin sheep (Dalga et al., 2018), while the length 25 (The greatest inner height of the orbit) and the length 27 (between the breadth of the occipital condyles) 26 (the greatest mastoid breadth (Otion-Otion)) there was a strong negative correlation.

CONCLUSION

As a result; This study is important because it is the first study on the head structure of one of Turkey's domestic sheep breeds. It is thought that the difference between the skulls of Konya merino and other sheep may be due to the breed of sheep. In addition, this research will contribute to the scientific studies to be carried out in this direction and to the literature on the subject.

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A comparative gross study on the Plexus Sacralis of the magpie (Pica pica) and chukar partridge (Alectoris chukar)

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ABSTRACT

Objective: The aim of the study is to compare the detection of plexus sacralis and its branches of Magpie (Pica pica) and Chukar partridge (Alectoris chukar) and to correlate the anatomic differences between these studied species.

Materials and Methods: In the study, 20 each magpies and Chukar partridges were used. Under anesthesia, the studied birds were sacrificed. Then all the nerves separating from the plexus sacralis were separately dissected and photographed

Results: In wingeds, plexus sacralis usually occurs as a result of a combination of ventral primary branches of 1-5 sacral spinal nerves. In the study, it was observed that formation of plexus sacralis and general anatomic distribution of nerves arising from plexus in both the species were similar with other species of birds. However, nerves forming the plexus sacralis and their branches were thicker in Chukar partridge than Magpie.

Conclusion: In conclusion, in the Magpie and Chukar partridge, anatomical distribution and nerve structure of plexus sacralis were determined, and it was observed that there was no major anatomical difference between them.

Keywords: *Chukar partridge, Magpie, Plexus sacralis*

INTRODUCTION

Magpies, living in the geography including Asia, Europe and North America, belong to Corvidae family. Magpies in Turkey is called " *Pica pica* " known as European Magpie (Hayman et al., 2005; Anonym, 2014). Partridges belong to the Pheasin (Phasinidae) family (Özçelik, 1995; Robbins, 1998; Kırıkçı and Çetin, 1999). They live in the region stretching from the Balkans to Central and Northwest Anatolia (Gaudioso et al., 2002).

Plexus sacralis in wingeds birds is formed by the combination of primary ventral branches of 1-5th sacral spinal nerve in three roots; truncus cranialis, truncus medianus and truncus caudalis (Baumel, 1975; Baumel et al., 1993). It is called "nervus (n.) bigeminus" which is the last of these branches forming plexus sacralis and the nerve is connected to plexus sacralis by distal of plexus pudendus through caudal branch (Baumel et al., 1993; Serbest et al., 1993; Dursun, 2002; El-Mahdy et al., 2010). Nervus gluteus caudalis, nervus cutaneus femoris caudalis, rami (rr.) musculares, nervus ischiadicus, nervus tibialis and nervus peroneus come out from plexus sacralis in domestic birds (Nickel et al., 1977; Dursun, 2002).

Nervus ischiadicus the thickest nerve of plexus sacralis and also named as “siatic nerve”, is divided into nervus peroneus (nervus fibularis) and nervus tibialis in the medial part of proximal of articulatio genu (Doğuer and Erençin, 1964; Nickel et al., 1977;

Baumel et al., 1993; Dursun, 2002). In rock partridge and Japanese quail n. peroneus proceeds to the distal part of femur with n. tibialis as a single stem. It separates from n. tibialis in the proximal of articulatio genu and after then divides into a slim branch n. peroneus superficialis and a thick branch n. peroneus profundus (Can, 2011).

In the literature, it is seen that although there are many scientific studies on the plexus sacralis in different animal species, there are not enough studies on the plexus sacralis in birds, especially wild bird. The aim of the study is to compare the plexus sacralis and its branches of Magpie and Chukar partridges and to investigate the variances in both studied species.

MATERIALS and METHODS

This study is according to the examination made by the Atatürk University Veterinary Faculty, Ethics Subcommittee (Decision no:2014/9). In the study, 20 Magpies and 20 Chukar partridges were used. Then, arteria carotis communis of each anesthetized Magpies and Chukar partridges was cut and blood was shed, an incision was made through the ventromedian line longitudionally in the abdominal region. The abdominal organs were taken out and were immersed in 10% formaldehyde solution for dissection. The nerves composing the plexus sacralis were dissected and photographed. The terms in Nomina Anatomica Avium (NAA) (Baumel et al., 2013) were used as base in the process of naming of the nerves and the surrounding anatomical formations.

RESULTS

Plexus Sacralis: It was determined that plexus sacralis in Magpie and Chukar partridge was formed as a result of union of primary ventral branches of 5 (5-9) synsacral spinal nerves. (Figure 1a). It was determined that ventral branches of 5th and 6th synsacral spinal nerves formed "truncus cranialis", 7th synsacral spinal nerve formed "truncus medianus" alone and ventral branches of 8th and 9th synsacral spinal nerves formed "truncus caudalis" (Figure 1b).

Nervus ischiadicus: It was seen that after its formation in pelvis, nervus ischiadicus progresses to caudolateral and passes through for. (foramen) ischiadicum. Nervus ischiadicus divided into four branches as common stem of nervus peroneus and nervus tibialis, nervus coxalis caudalis, nervus cutaneus femoris caudalis and rami musculares (Figure 2c-d).

Nervus coxalis caudalis: In both the species under this study, it was observed that it proceeds to cranioventral shortly after leaving n. ischiadicus in line of foramen ischiadicum and innervates the anterior part of m. (musculus) biceps femoris giving two branches (Figure 2c-d).

The Common Stem of nervus peroneus and nervus tibialis: It was observed that this common stem was the thickest branch among the branches given by n. ischiadicus in Magpie. When it reached medial aspect of the femur it divided into n. peroneus and n. tibialis between the 1/3 inferior of femur and the proximal part of articulatio genu (Figure 2a-c, Figure 2c). It was observed that in Chukar partridge, these two nerves (nervus peroneus and nervus tibialis), which separated from the same root, continued independently of each other in the lower 1/3 of the femur. The nerve located anteriorly and surrounded by a weak sheath was the nervus peroneus, while the posterior nerve was the nervus tibialis. It was observed that the nervus cutaneus suralis was separated from the nervus tibialis (Figure 2b-d, Figure 2d).

Nervus cutaneus suralis: It was observed that in Magpie n. cutaneous suralis originated from the common stem of n. peroneus and n. tibialis (Figure 2a). In Chukar partridge n. cutaneous suralis separated from n. tibialis at the medial aspect of the femur after n. tibialis and n. peroneus separated from each other (Figure 2b).

Nervus peroneus: It was seen that in Magpie n. peroneus gave a thin branch n. paraperoneus shortly after, n. peroneus and tendons of m. biceps femoris, pass from ansa mm. (musculi) iliofibularis at the caudolateral of articulatio genu proceeding to cranial after it separated from n. tibialis. It was determined that n. peroneus gave thin muscular branches at the underside of for. interosseum proximale and then proceeded to the distal part of leg. It was determined that it divided into two main branches as nervus peroneus superficialis and nervus peroneus profundus at 1/2 of ossa cruris (Figure 1c, 1e). It was determined that in Chukar partridge n. peroneus divided into n. peroneus profundus after it gave thin muscular branches whose numbers differed between 2-3 in the direction of cranial in the lateral aspect of proximal part of leg on os fibula turned to craniolateral leaving n. paraperoneus in the proximal part of ossa cruris shortly after it passed from ansa mm. iliofibularis in the caudolateral of art. (articulatio) genu.

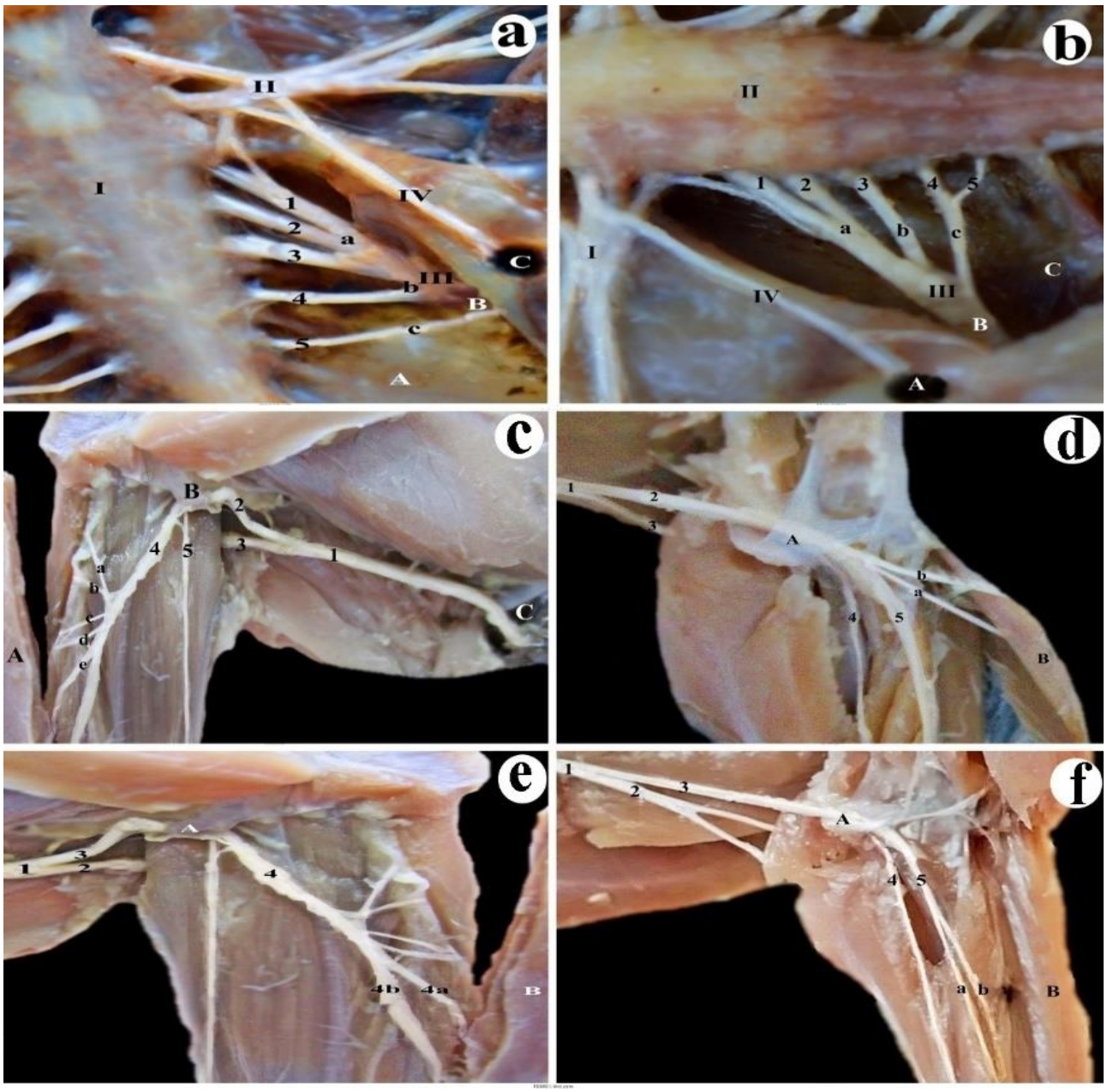

Figure 1. Gross anatomical views of plexus sacralis, n. peroneus, its branches and n. peroneus superficialis et profundus;

a: Plexus sacralis and formation of n. ischiadicus in Magpie, left caudoventral view; *I: Synsacrum, II: Plexus lumbalis, III: Plexus sacralis, IV: N. obturatorius, 1-5: Ventral branches of 5-9. synsacral spinal nerves, a: Truncus cranialis, b: Truncus medianus, c: Truncus caudalis, A: Fossa renalis caudalis, B: N. ischiadicus, C: Foramen obturatum.*

b: Plexus sacralis and formation of n. ischiadicus in Chukar partridge, left ventrolateral view; *I: Plexus lumbalis, II: Synsacrum, III: Plexus Sacralis, IV: N. obturatorius, 1-5: Ventral branches of 5-9. synsacral spinal nerves, a: Truncus cranialis, b: Truncus medianus, c: Truncus caudalis, A: Foramen obturatum, B: N. ischiadicus, C: Fossa renalis caudalis.*

c: N. peroneus and its branches in Magpie, left lateral view; *1: The common stem of n. peroneus ve n. tibialis, 2, 4: N. peroneus, 3: N. tibialis, 5: N. paraperoneus, a-d: Muscular branches of n. peroneus, e: N. peroneus superficialis, A: M. tibialis cranialis, B: Ansa mm. İliofibularis, C: Foramen ischiadicum.*

d: N. peroneus and its branches in Chukar partridge, right lateral view; *1: The common stem of n. peroneus ve n. tibialis, 2, 5: N. peroneus, 3: N. tibialis, 4: N. paraperoneus, a, b: Muscular branches of n. peroneus, e: N. peroneus superficialis, A: Ansa mm. İliofibularis, B: M. tibialis cranialis.*

e: N. peroneus superficialis et profundus in Magpie, right lateral view; *1: The common stem of n. peroneus ve n. tibialis, 2: N. tibialis, 3, 4: N. peroneus, 4a: N. peroneus profundus, 4b: N. peroneus superficialis, A: Ansa mm. İliofibularis, B: M. tibialis cranialis.*

f: N. peroneus superficialis et profundus in Chukar partridge, right lateral view; *1: The common stem of n. peroneus ve n. tibialis, 2: N. tibialis, 3, 5: N. peroneus, 4: N. paraperoneus, a: N. peroneus superficialis, b: N. peroneus profundus, A: Ansa mm. İliofibularis, B: M. tibialis cranialis.*

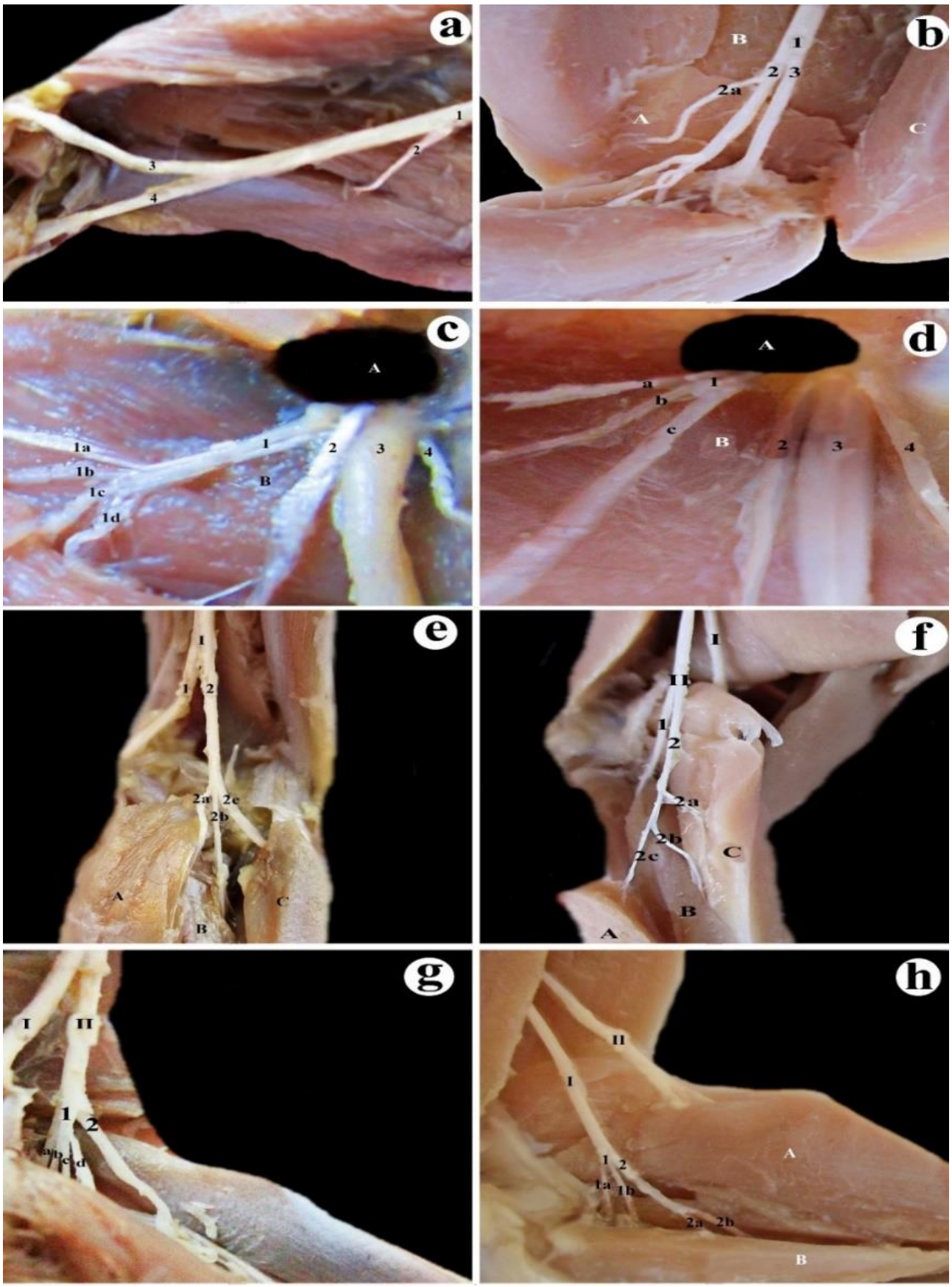

Figure 2. Gross anatomical views of n. cutaneus suralis, rami musculares, n. tibialis, its branches and n. tibialis lateralis et medialis;

a: N. cutaneus suralis in Magpie, left lateral view; *1: The common stem of n. peroneus ve n. tibialis, 2: N. cutaneus suralis, 3: N. peroneus, 4: N. tibialis.*

b: N. cutaneus suralis in Chukar partridge, right lateral view; *1: The common stem of n. peroneus ve n. tibialis, 2: N. tibialis, 2a: N. cutaneus suralis, 3: N. peroneus, A: M. puboischiofemoralis lateralis, B: M. caudofemoralis, C: M. biceps femoris.*

c: N. ischiadicus and its branches in Magpie, right lateral view; *1: Rr. musculares, 1a, 1b, 1c, 1d: Branches of rr. musculares, 2: N. cutaneus femoris caudalis, 3: The common stem of n. peroneus ve n. tibialis, 4. N. coxalis caudalis, A: Foramen ischiadicum, B: M. ischiofemoralis.*

d: N. ischiadicus and its branches in Chukar partridge, right lateral view; *1: Rr. musculares, a, b, c: Branches of rr. musculares, 2: N. cutaneus femoris caudalis, 3: The common stem of n. peroneus ve n. tibialis, 4: N. coxalis caudalis, A: Foramen ischiadicum, B: M. ischiofemoralis.*

e: N. tibialis lateralis and its branches in Magpie, left caudolateral lateral view; *I: N. tibialis, 1: N.tibialis medialis, 2: N. tibialis lateralis, 2a, 2b, 2c: The muscular branches of n. tibialis lateralis, A: M. flexor perforans et perforatus digiti III, B: M. flexor perforans et perforatus digiti II, C: M. gastrocnemius pars lateralis.*

f: N. tibialis lateralis and its branches in Chukar partridge, right caudolateral lateral view; *I: N. peroneus, II: N.tibialis, 1: N. tibialis medialis, 2: N. tibialis lateralis, 2a, 2b, 2c: The muscular branches of n. tibialis lateralis, A: M. flexor perforans et perforatus digiti III, B: M. flexor perforans et perforatus digiti II, C: M. gastrocnemius pars lateralis.*

g: N. tibialis medialis and its branches in Magpie, left lateral lateral view; *I: N. tibialis lateralis, II: N.tibialis medialis, 1: The cranial branc of n. tibialis medialis, 2: The caudal branch of n. tibialis medialis, a-d: The muscular branches of n. tibialis medialis cranialis.*

h: N. tibialis medialis and its branches in Chukar partridge, right lateral lateral view; *I: N. tibialis medialis, II: N.tibialis lateralis, 1: The proximal branch of n. tibialis medialis, 1a, 1b: The muscular branches, 2: The caudadistal branch of n. tibialis medialis, 2a, 2b: The muscular branches, A: M. gastrocnemius pars lateralis. B: M. gastrocnemius pars medialis.*

It was determined that in Chukar partridge thin muscular branches separated from n. peroneus to cranial at the lateral aspect of the upper part of leg, then one of two main branches took part in the caudal proceeding to distal was n. peroneus superficialis. It was detected that it took part as n. metatarsalis dorsalis lateralis on upside of leg passed from retinaculum extensorium tibiotarsi (Figure 1d, 1f).

Nervus tibialis: It was determined that in Magpie n. tibialis separated from the common stem at inferior 1/3 of femur. Then it ran towards caudal of art. genu and divided into two main branches going towards medial and lateral at the dorsal of ansa mm. iliofibularis, the branch going towards lateral was n. tibialis (suralis) lateralis and the thick branch going towards medial was n. tibialis (suralis) medialis (Figure 2e, 2g).

It was seen that in Chukar partridge nervus tibialis separated from the common stem of n. tibialis and nervus peroneus at 1/2 of the femur gave a thin branch nervus cutaneus suralis. It was observed that nervus tibialis divided into two branches as nervus tibialis medialis and nervus tibialis lateralis at inferior 1/3 of the femur (Figure 2f, 2h). Nervus tibialis lateralis divided into three branches (Figure 2f). It was seen that nervus tibialis medialis in proximal portion divided into two branches, proceeded cranially on the medial part of leg, the other branch of it separated towards caudodistal divided into two branches and dispersed in the caudal part of leg (Figure 2h).

In the study anatomical distributions and nerve structures of plexus sacralis belonged to Magpie and Chukar partridge species were determined and it has been revealed that there were some anatomical differences between them. The anatomical differences between Magpie and Chukar partridge whose plexus sacralis researched and with other species could be listed as follows:

- Truncus cranialis was formed by ventral branches of 5th and 6th synsacral spinal nerves in the two species, truncus medianus was formed by ventral branches of 7th and 8th synsacral spinal nerves in Magpie and ventral branches of 7th synsacral spinal nerve in Chukar partridge, truncus caudalis was formed by ventral branches of only 9th synsacral spinal nerve in Magpie and ventral branches of 8th and 9th synsacral spinal nerves in Chukar partridge.

- Nervus cutaneus suralis originated from common stem of nervus peroneus and nervus tibialis in Magpie and from nervus tibialis in Chukar partridge.

DISCUSSION

It was reported that this number is generally 6 in domestic birds (Nickel et al., 1977; Dursun, 2002) and white turkey (Istanbullugil, 2008), 5 in hen (Serbest et al., 1993), 5 or 6 in turkey (Serbest, 2000), 5 in goose (Serbest, 2000), 5 in owl (Akbulut et al., 2015). As the obtained data are partially similar to the literature information.

Can and Özdemir (2012) in rock partridge, Serbest et al. (1993) in hen, Akbulut and et al. (2015) in owl, reported that ventral branches of the first two synsacral spinal nerves in cranial participates in the formation of plexus sacralis composed the truncus cranialis, the third branch alone composed truncus medianus, the fourth and fifth branches together composed truncus caudalis.

It was reported that this nerve is formed by the combination of ventral branches of the first four

synsacral spinal nerves composing the plexus sacralis in domestic birds (Nickel et al., 1977; Martin et al., 1994; Dursun, 2002). İstanbullugil (2008) and Can (2011) reported that the second branch separates from n. ischiadicus is n. coxalis caudalis and it proceeds to craniocaudal.

It was determined that in the study unlike the literature in Magpie n. coxalis caudalis exits from dorsocranial of common stem of n. peroneus and n. tibialis, in Chukar partridge it exits from cranial of this stem and they innervate the cranial part of m. biceps femoris as two branches (Doğuer and Erençin, 1964; Istanbullugil, 2008; Can, 2011).

The information that in domestic wingeds (Nickel et al., 1977; Dursun, 2002), owl (Akbulut et al., 2015), Japanese quail (Can and Özdemir, 2011) and rock partridge (Can and Özdemir, 2012) the nerve that is one of the nerves composing the common stem of n. peroneus and n. tibialis is n. peroneus in cranial and the one in caudal is n. tibialis is similar to study findings.

Baumel (1975) reported that n. cutaneous suralis separates from n. tibialis in the medial of femur and innervate the region skin proceeding to distal as in Chukar partridge, Akbulut and et al. (2015) reported that in owl the mentioned nerve separates from n. tibialis side of common stem at 2-2.5 cm above of separating point of n. tibialis and n. peroneus.

It was determined that this nerve separates from caudal part of plexus lumbosacralis according to Jungherr et al. (1969) from plexus sacralis according to Dursun (2002), Nickel et al. (1977), from common stem of n. tibialis and n. peroneus in the same cover with n. paraperoneus according to El- Mahdy et al. (2010) and Akbulut et al. (2015).

El-Mahdy et al. (2010) in ostrich, Balkaya and Özüdoğru (2016) in sparrow hawk determined that, like the findings in Magpie, n. peroneus gives muscular branches innervating musculus tibialis cranialis, musculus fibularis longus, musculus extensor digitorum longus in the proximal part of leg and divides into n. peroneus superficialis et profundus after proceeding to distal on the lateral surface of leg. Similar to domestic birds (Nickel et al., 1977; Dursun, 2002) n. peroneus composes n. peroneus superficialis et profundus after it gives thin muscular branches in Chukar partridge. It was seen that some findings found in the study are not similar to some studies (Nickel et al., 1977; Dursun, 2002). Can (2011) reported that n. peroneus profundus divides into 4 branches in Japanese quail and 5 in rock partridge and İstanbullugil (2008) reported that it divides into 6 in white turkey. On the other hand, it was observed that differently from literature findings (Fitzgerald, 1969; Nickel et al., 1977; Istanbullugil, 2008¸Bentley and Poole, 2009; El-Mahdy et al., 2010) n. peroneus profundus is seen prominently in Magpie and visits lateral and medial bifurcation after passes through retinaculum extensorium tibiotarsi.

Some researchers (Nickel et al., 1977; Dursun, 2002) reported that in wingeds n. tibialis gives r. (ramus) lateralis and r. medialis and these two branches innervate popliteal region, m. gastrocnemius and the flexor muscles on the posterior surface of calf. It has been reported that the first branch given by nervus tibialis before n. tibialis lateralis divides into et medialis in sparrow hawk (Balkaya, 2012), pigeon (Balkaya, 2012), Japanese quail (Can and Özdemir, 2011), rock partridge (Can and Özdemir, 2012) and ostrich (El-Mahdy et al., 2010) is n. paraperoneus.

CONCLUSION

In conclusion, in the Magpie and Chukar partridge, anatomical distribution and nerve structure of plexus sacralis were determined, and it was observed that there was no major anatomical difference between them. It has been thought that this study will contribute the scientific studies to be done in this direction and the literature related to the subject.

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Sero-epidemiology of bovine tuberculosis in dairy cattle in Chattogram, Bangladesh

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ABSTRACT

Objective: Bovine tuberculosis (bTB) is caused by *Mycobacterium bovis* both in wild and domesticated animals including cattle, and is a significant public health concern due to its cross-species transmissibility. We conducted this study on the dairy farms in Chattogram district of Bangladesh to estimate the seroprevalence and potential risk factors at both animal and farm levels associated with the occurrence of bTB. We targeted to illustrate a complete picture of bTB to the farmers, policymakers and dairy practitioners.

Materials and Methods: Based on the highest density of intensive dairy cattle farms, we recruited three subdistricts namely Double Mooring, Shikolbaha, and Raozan of Chattogram for this cross-sectional study. We sampled a total of 538 animals from randomly selected 37 farms of the selected subdistricts. We collected blood samples from the animals for performing ELISA in the laboratory and used a pretested questionnaire for data collection and epidemiological analysis.

Results: We estimated the overall seroprevalence of bTB was 38.2% and 7.5% at the farm level and animal level respectively. Random effect logistic regression model estimated the low to moderate stocking density (OR=19.6, p=0.02) as the significant risk factor of bTB at the farm level whereas, farms own stock (OR= 3.4, p=<0.01) has been calculated as significant risk factors at individual animal level.

Conclusion: For a dairy-intensified area of any developing country like Bangladesh, a coordinated effort of both veterinarians and local public health officials is critical for implementing an efficient TB control program. A comprehensive survey is always recommended for early detection and control of the zoonotic spillover events of any organisms. Therefore, these research findings will aid in the prevention and control of bTB in the studied region and will prompt removal and good farm management practices. Overall, this study will make dairy farmers and policy planners aware of the necessity of continuous surveillance to eradicate TB from the farm levels in any developing and underdeveloped nations across the world.

Keywords: *Tuberculin, bTB, Prevalence, Dairy farming, Risk factor, ELISA*

INTRODUCTION

Mycobacterium bovis causes bovine tuberculosis (bTB), which is a major zoonotic disease worldwide (Proano-Perez et al., 2006). The disease is most commonly found in cattle, although it can also be found in other domestic animals, wildlife, and humans (Filia et al., 2016). Humans are infected mostly through inhalation of aerosols created by infected animals, as well as intake of raw, unpasteurized milk (Thakur et al., 2012). The dairy

business is a top priority for emerging Asian, African, Latin American, and Caribbean countries, including Bangladesh. Due to a lack of adequate management techniques, the dairy industry's intensification is promoting bTB transmission (Proano-Perez et al., 2006). The disease is rising in many parts of the world especially in Asia and Africa(Collins, 1993; Ameni et al., 2003). This is due to lack of organized and practicable test methods for mass screening (Asiak et al., 2007). Due to its substantial economic impact on animal production and zoonotic nature, bTB has been a serious public health concern for the past three decades.

Seroprevalence of bovine tuberculosis at various levels (cattle and farm) varies widely over the world. The reported prevalence of bTB seroprevalence ranged from 5.9% to 30% (Rahman and Samad, 2008; Mahmud et al., 2014; Mondal et al., 2014; Chakraborty et al., 2015). Many research have been conducted around the world to establish the risk variables related with bTB seropositivity. BCS (poor) (OR=4.4), parity (4 calving) (OR=2.3), history of coughing (OR=6.7), and bigger herd size (OR=5.9) were found to be significant risk factors for bTB in cattle in a prior study in Bangladesh (Mondal et al., 2014; Chakraborty et al., 2015). Rapid removal of infected animals is critical for limiting transmission, and tuberculin skin tests can effectively diagnose early *Mycobacterium bovis* infection in cattle(Buddle et al., 2009). Infected animals are occasionally anergic to the skin test in the late stages of disease due to increased humoral antibodies (Lilenbaum et al., 1999). Bovine tuberculosis diagnosis by antibody based tests are using for many years (Pollock et al., 2001)and it is used to identify anergic cows (Janeiro, 2006). The antibodies are usually found only in later stages of disease and recently infected animals will not react to antibody based test (Wahlström, 2004).The use of ELISA as a beneficial supplemental tool in field conditions for the control of bovine tuberculosis has been demonstrated in practice (Janeiro, 2006).The diagnosis of bovine tuberculosis is a complicated process that relies on a range of laboratory techniques, including serological assays. Traditional ELISA is used for serological testing (Sensitivity and Specificity are 83.2-93.1% and 86.5-98.4%respectively) (Lilenbaum et al., 1999; Lilenbaum et al., 2001; Whelan et al., 2008; Souza et al., 2012) are most frequently used.

Bangladesh is one of the world's most densely inhabited countries, with people living in close proximity to their animals. As a result, the transmission of bTB infection from animals to humans is quite likely. Because of the lack of a monitoring program, limited diagnostic facilities, and the lack of veterinary inspection in slaughterhouses, the status of bTB in Bangladesh remains unclear. On this background this study aimed to estimate the animal and farm level seroprevalence of bTB based on enzyme linked immunosorbent assay and to identify the associated risk factors in order to develop effective bTB prevention and control techniques.

MATERIALS and METHODS

This study was approved by the ethics committee of Chattogram Veterinary and Animal Sciences University and with the decision number CVASU/Dir (R&E) EC/2023/500 (6).

Description of the study areas

For this study, three key dairy cattle areas in Chattogram were purposefully chosen. They were: 1) Double Mooring (Urban), 2) Shikalbaha (Peri-urban), and 3) Raozan (Peri-urban) (Rural). Between 22°18' and 22°21' N latitudes and 91°48' and 91°51' E longitudes, Double Mooring is an important portion of the Chattogram metropolitan region, located alongside the Bay of Bengal. In comparison to other metropolitan areas, it has the highest number of intensive dairy farms with high yielding cross breeds (N=415) (DLO, DLS of Chattogram, Personal Communication, 2018). It is the city's main source of milk. Shikalbaha has the most dairy cattle farms (N=400) of all the peri-urban communities in the Chattogram metropolitan area (DLO, DLS of Chattogram, Personal Communication, 2018). It lies between 22'11' and 22'24' N latitudes and 91'48' and 91'52' E longitudes, alongside the river Karnaphuli (Anon, 2022). Because this location has excellent road and water connections to various parts of Chattogram, a variety of companies have sprung up, including a Dairy Milk Processing Plant. Agriculture is also a significant source of income for the residents of this area. Raozan is 32 kilometers from the Chattogram metropolitan region, with latitudes of 22°25' and 22°40' N and longitudes of 91°51' and 91°59' E. (Anon, 2022). It is bordered on the south by the Karnaphuli River and on the west by the Halda River. Road and water communication are excellent. It has a large number of intensive dairy farms (N=150) with exotic breeds that produce excellent yields (DLO, DLS of Chattogram, Personal Communication, 2018). In Figure 1, we depicted the

study area's geographical position using ArcGIS software 10.8 (Sayeed et al., 2020a).

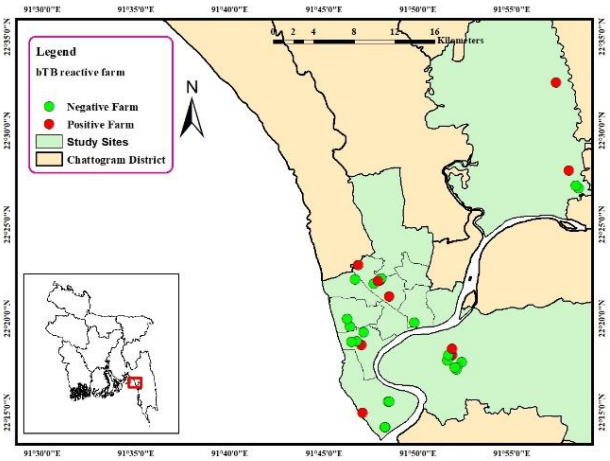

Figure 1. Map bTB reactive study farm.

Study population, duration and study design

A bTB sero-survey was conducted in dairy cattle of high yielding exotic breed in Chattogram during May 2014 to July 2014.Commercial dairy farms and dairy cattle of Chattogram district were considered as a reference population. Dairy farms and dairy cattle of three study areas of Chattogram were considered as a source population. A farm consisting of at least 15 exotic cattle breed was defined as the smallest sampling unit. Accordingly, a total of 96 farms were enlisted among which 65, 21 and 10 farms were belonged to Double Mooring, Shikolbaha and Raozan, respectively.

Sample size calculation and sampling

A total of 41 farms were required assuming 7% farm level bTB prevalence (Noorrahim et al., 2015) with ±5% precision, 90% confidence interval and 1.0% design effect (http://www.openepi.com/Menu/OE_Menu.htm). However, employing a proportionate chance of random sampling with some variance, 37 farms (90% response rate) were recruited. As a result, 22 farms in Double Mooring (eligible=538, total animal=959), 10 in Shikolbaha (eligible=200, total animal=307), and 5 in Raozan (eligible=108, total animal=204) were distributed. Individual animals aged 6 months or longer were regarded suitable for this study's bTB investigation. The chance of an individual animal exposing bTB increased with age, according to past research carried out in Tanzania and Uganda (Cleaveland et al., 2007; Inangolet et al., 2008), and in Bangladesh, cases of bTB in young animals (under 6 months old) were not being reported (Mahmud et al., 2014b) hence we considered those animals for sampling that were older than 6 months As a result, all animals that satisfied the selection criteria were included in the study, with a total of 538 animals in Double Mooring, 200 in Shikolbaha, and 108 in Raozan.

Collection of blood samples

ELISA was performed on 52% of first tuberculin test (CFTT) cattle, with typical collection ranges of 25-93% of CFTT treated animals across farms. We also performed the ELISA on 34 of the 37 CFTT farms. Due to farmer non-cooperation and technical difficulties, three farms were overlooked. We collected a total of 442 blood samples from 34 farms. Five (5) mL blood was extracted aseptically from the jugular vein of each animal and transferred to a sterile vacutainer (without anticoagulant) labeled with a unique identifying number. Within 2-3 hours of collection, the samples were transported to Chattogram Veterinary and Animal Sciences University's Physiology and Biochemistry laboratory. The whole blood sample was spun at 3000 RPM for 30 minutes to separate the serum. The samples were then kept in the laboratory at -20°C until they were tested at the Poultry Research and Training Centre's laboratory (PRTC).

Recording of data

Face-to-face questionnaire interviews and physical observations were used to collect baseline and risk factor information at the farm and animal level. Each interview and observation lasted around an hour, and each farmer's verbal consent was obtained prior to the commencement of the interview. Farm-level data is made of farmer and farm address, farm coordinates, farmer's education, farmer's knowledge about bTB, farm establishment date, farm size, farm house type, stocking density, floor type, ventilation status, farm sanitary condition, farm biosecurity, mixed with other animals and feeding system. Animal level information consisted of breed, sex, age, parity, body condition score (BCS), lactation status, milk production, pregnancy status and source of animal.

Variable measurement

Farm coordinate information was taken using GIS machine (Model no: eTrex 10 and Company: Garmin, China). Risk factors were classified based on standard essential facilities of a dairy farm. Potential risk factors both in animal related and farm related were recorded by an organized questionnaire and data entry sheet. Collected animal related risk factors were age, breed, sex, parity, body weight, BCS, lactation and pregnancy status, sources of animals etc. and farm establishment date, total population, cattle

movement, previous history about PPD, source of semen, farm management system etc. were collected both by asking the owner and direct observations. Personal information on TB was also gathered by asking owners about their educational level, understanding of bTB (specifically, how it is transmitted, symptoms, etc.), and history of TB in farm workers or family members, as well as contact with animals.

Laboratory evaluation

This study used the AniGen BTB Ab ELISA test kit from BioNote, Inc., 2-9, Seogu-dong, Hwaseong-si, Gyeonggi-do, Korea (445-170). The test kit was kept between 2 and 8 degrees Celsius until it was used. The PRTC Laboratory at Chattogram Veterinary and Animal Sciences University examined serum samples for ELISA.

Data entry and statistical evaluation

Data generated from the study were entered into the spreadsheets of Microsoft Excel 2007 program. Data were then cleaned, coded, and checked for integrity before exporting to STATA-IC13 (Stata Crop, 4905, Lakeway Drive, College station, Texas 77845, USA) for epidemiological analysis.

Descriptive analysis

We calculated the bTB seroprevalence based on ELISA results. A bTB sero-positive farm was considered as a positive farm when a farm had at least one animal tested reactive to ELISA. Bovine tuberculosis sero-positive and negative farms were displayed according to farms and areas using the respective coordinate information and ArcGIS software Version 10.2.1 (Sayeed et al., 2020a). We computed the seroprevalence of bTB at animal level using the total number of ELISA reactive animals divided by the total number of animals tested. The Fisher's exact test and Chi-Square test was performed between categories of the selected factors and a binary response variable for bTB ELISA result at farm/animal level (Yes/No) to assess differences in the proportion of bTB sero-positives between categories of each factor. The results were expressed in a frequency number and a percentage along with 95% confidence interval. The level of significance was set at 0.05 or less.

Risk factors analysis

A set of demographic and management related variables recorded using questionnaire were considered for the risk factor analysis of bTB sero-prevalence. Risk factor analysis for bTB seroprevalence at farm level and animal level were done by random effect logistic regression analysis (Sayeed et al., 2017) where "Farm ID was accounted as a cluster variable". In animal level, significant factors ($p \leq 0.30$) at the univariate *Chi-Square* test were moved to multivariate analysis and in farm level; significant factors ($p \leq 0.30$) at the univariate Fisher's exact test were moved to multivariate analysis. We have used the standard model building procedure along with the checking of confounding and interaction variables, and independency among independent variables as well as validity of the model was explained as described by (Sayeed et al., 2020b). We presented the results as odds ratio (OR), Standard Error (SE), 95% Confidence Interval (CI), and p value.

RESULTS

Seroprevalence estimates of bTB

The seroprevalence of bTB was 38.2% (95% CI: 21.1-52.4) at farm level and 7.5% (95% CI: 4.4-12.3%) at animal level (Table 1).

Table 1. Overall seroprevalence of bovine tuberculosis (*Mycobacterium bovis*) in dairy cattle of Chattogram district (N=442).

Level	No of units tested	No of positive units (%)	95% CI
Farm	34	13 (38.2)	21.1-52.4
Individual level (Farm as cluster)	442	33 (7.5)	4.4-12.3
Individual level (Ignoring farm as cluster)	442	33 (7.5)	5.3-10.3

Risk factors of bTB

Univariate association between factors and bTB seroprevalence at farm level

The farm level bTB seroprevalence was significantly higher ($p=0.03$) in case of larger farm size (N=33-160) than the smaller farms size (N=1-32) (Table 2). Farms belonging to Double Mooring, older farms, face-in housing system, low or moderately populated farms, less frequent contact between animals and humans and farms of poor biosecurity standard, irrespective of kind of prevalence, had greater bTB prevalence, but the association was statistically insignificant ($p<0.05$) (Table 2).

Univariate association between factors and bTB seroprevalence at animal level

Animal level bTB seroprevalence significantly higher in case of own stock (*P*=0.001) than the purchased stock. Others factors including BCS, parity, lactation status, pregnancy etc. are statistically insignificant (Table 3).

Risk factors analysis

Farm level risk factors analysis (random effect regression model)

Farm size with larger stocking (N=33-160) has significantly higher (OR=26.2) risk for bTB than the smaller farm size (N=15-32). Beside this, farms with low/moderate stocking density has significantly higher (OR=19.6) bTB infection than the optimum/high stocking density (Table 4).

Table 2. Univariate association between factors and bTB seroprevalence at farm level in Chattogram, Bangladesh (Fisher's Exact Test)

Factor	Category	ELISA		
		+ n (%)	-n	p
Area	Urban	8 (40.0)	12	1.08
	Peri-urban	5 (35.7)	9	
Farm size/population size	Min-32	3 (17.7)	14	**0.03**
	33-max	10 (58.8)	7	
Establishment year	1980-2000	8 (47.1)	9	0.48
	2001-2013	5 (29.4)	12	
Housing	Face in	9 (52.9)	8	0.16
	Face out	4 (23.5)	13	
Floor type	Concrete	10 (38.5)	16	1.30
	Herring bone	3 (37.5)	5	
Ventilation	Poor/Fair	6 (33.30)	12	0.79
	Good	7 (43.8)	9	
Stocking density	Low/Moderate	10 (41.7)	11	0.29
	Optimum/High	3 (23.1)	10	
Sanitary Condition	Poor/fair	10 (43.5)	13	0.60
	Good	3 (27.3)	8	
Contract between human and other animal	Minimum	7 (41.2)	10	1.11
	Moderate/Intimate	6 (37.5)	10	
Mixed with other animals	No, not all	10 (37.1)	17	1.10
	Yes, (sometimes with other animal species of the farmers)	3 (42.9)	4	
Feeding	Separate	11 (36.7)	19	1.00
	Common/both	2 (50.0)	2	
Biosecurity	Poor	5 (50.0)	5	0.60
	Fair/Good	8 (33.3)	16	
Education of the farmer	Illiterate to HSC	5 (33.3)	10	0.87
	Graduate and or more	8 (42.1)	11	
Knowledge about TB	Yes	8 (30.8)	18	0.40
	No	4 (57.1)	3	

Table 3. Univariate association between factors and sero-bTB at animal level in Chattogram, Bangladesh (*Chi-Square* Test).

Factor	Category	ELISA		P (*Chi-Square* Test)
		+ n (%)	-n	
Age	Min/66	15 (6.5)	216	0.34
	68/max	18 (8.9)	184	
Parity	Min/2	7 (5.2)	128	0.43
	3	10 (9.1)	100	
	4/Max	14 (8.6)	149	
BCS	Min/3	18 (8.5)	195	0.45
	3.5/4	15 (6.6)	214	
Lactation	No	16 (6.6)	228	0.42
	Yes	16 (8.6)	170	
Pregnancy	No	16 (6.6)	228	0.42
	Yes	16 (8.6)	170	
Source	Own stock	17(14.4)	101	**0.001**
	Purchased	16 (5.1)	299	
Milk production	Min/16 litter	16 (6.7)	214	0.56
	17/Max litter	15 (8.5)	161	
Farm area	Urban	8 (40.0)	12	1.08
	Peri-Urban/Rural	5 (35.7)	9	

Table 4. Outputs of random effect model (at farm level).

Factor	Category	OR	95% CI	p value
Farm size/population size	15-32	1.0		
	33-160	26.2	2.2-319.1	0.01
Housing	Face out	1.0		
	Face in	4.5	0.7-30.0	0.123
Stocking density	Optimum/High	1.0		
	Low/Moderate	19.6	1.5-261.5	0.024

Table 5. Outputs of the random effect model (at animal level).

Factor	Category	OR	95% CI	p value
Source	Purchased	1.0		
	Own stock	3.4	1.4-8.1	0.006
Age	Min/66	1.0		
	66/max	1.8	0.8-4.0	0.127

Animal level risk factors analysis (random effect regression model)

Animal from own source has a significantly higher (OR=3.4) bTB infection than the animal from purchased source in the farms (Table 5).

DISCUSSION

Bovine tuberculosis is one of the important zoonotic diseases and a serious public health concern all over the world (Proaño-Perez et al., 2006; Javed et al., 2010; Awah-Ndukum et al., 2012). Bangladesh, in particular, is one of the densely populated countries in the world and peoples live very closely with their domestic animals. Therefore, likely transmission of bTB infection between animals and humans can easily be occurred in Bangladesh context. So, sero-epidemiological exploration of bTB was warranted to identify appropriate strategies to prevent and control bTB. This section discusses the important

findings of the current study on sero-epidemiology bTB and their implications as well as potential limitations.

In the current investigation, farm-level bTB seroprevalence was high (38%) but could not be compared due to a lack of comparable national and international records. Other Bangladeshi research back up the current study's 7.5% animal (cross-bred) level bTB sero-prevalence: 7.9%in a combination Red Chattogram cattle and Holstein Friesian in Chattogram (Chakraborty et al., 2015), 7.9% in Holstein Friesian in Sirajganj (Mahmud et al., 2014), 5.9% in Holstein Friesian in Mymensingh (Mondal et al., 2014) and5.88% in crossbred cattle of Chattogram metropolitan area (Chakraborty and Prodhan, 2020). However, some previous studies have found greater levels of bTB sero-prevalence, such as 30% in Mymensingh's Red Chattogram cattle (Rahman and Samad, 2008). Different countries had varying levels of bTB seroprevalence in cattle. In India, estimates of bTB seroprevalence ranged from 3.2-13.8% (Prakash et al., 2015; Didugu et al., 2016), 1.4% in Albania (Koni et al., 2015), 1.0% in Lao People's Democratic Republic (Vongxay et al., 2012), 10.4% in Ethiopia (Ameni et al., 2010), 37.2% in Cameroon (Awah-Ndukum et al., 2012), 36.3% in Nigeria (Asiak et al., 2007), 3.49% in east Algeria (Djafar et al., 2020) and 34.38% in Pakistan (Leghari et al., 2020).Geographical differences breed types, and management approaches could explain the disparities in bTB seroprevalence in the cited references (Omer et al., 2001; Lilenbaum et al., 2007; Nuru et al., 2015; Endalew et al., 2017).

Variables at the individual animal and farm level were studied to better understand the disease's epidemiology. By using a random effect model, the source of cattle (own stock: OR=3.4, 95% CI: 1.4-8.1, p=0.006) was found as a possible risk factor at the individual animal level. The potentiated risk factors by random effect model were population size (Larger: OR=26.2, 95% CI: 2.2-319.1, p=0.010) and stocking density (Low or moderate: OR=19.6, 95% CI: 1.5-261.5, p=0.024). International cattle movement or purchase has been recognized as a herd-level risk factor (Tschopp et al., 2009; Singhla et al., 2017). Animals were classified into two categories in this study: own stock and purchase. Surprisingly, purchased animals had a lower prevalence of bTB infection than own-stock cattle in this study. Although, an author reported that purchased cattle (Tschopp et al., 2009a) is a risk factor for bTB on the farm the difference between the earlier findings with our findings might be due to the purchasing of new cattle from low bTB risk areas (Sedighi and Varga, 2021), other factors including the difference includes the husbandry practice and geographical area of the study. The procurement of livestock from non-endemic areas could be one of the explanations (Dejene et al., 2016) or less endemic areas from this study. As an aerosol transmitting disease, close contact between animals is an important risk factor for bovine tuberculosis (Ameni et al., 2006). Unfortunately, in this study, farms with low and moderate animal density had a higher incidence than farms with optimal and high animal density. Because of the small number of reactor farms, some critical risk considerations may be overlooked.

By random effect model, population size is a potential risk factor in this study, which is consistent with some prior studies from various nations including Bangladesh, (Islam et al., 2020; Islam et al., 2021); Ecuador, (Proano-Perez et al., 2006; Proaño Pérez et al., 2009); Eritrea, (Omer et al., 2001); Zambia, (Cook et al., 1996); Tanzania, (Cleaveland et al., 2007); Ethiopia, (Ameni et al., 2003); Nigeria, (Ibrahim et al., 2010); Ethiopia, (Ameni and Erkihun, 2007). In case of larger herd, the risk of introduction of infected animal also become high (Cleaveland et al., 2007; Cadmus et al., 2010). The dairy farms those are not under bTB control measures, one infected cattle can transmit the disease to 2.2 cattle per year and this was calculated from Argentina (De Kantor and Ritacco, 2006). *Mycobacterium bovis* transmission mainly occurs through aerosols (Skuce et al., 2012)which depends on the density of animals (Huang et al., 2013) and especially in intensive farming practices this is more appropriate in larger farms than smaller. Unfortunately, in this investigation, stocking density (low or moderate) was found as a potential risk factor. It could be because there are fewer seropositive farms.

The power of this study was not so high because only 13 farms were found as a reactor farms and some important risk factors in farm level may have been missed. In individual cattle level, eight (8) variables were analyzed but only source of animals (own stock/ purchased) was found as a potential risk factors (p=0.001). Due to minimum number of infected cattle (33), some important risk factors in cattle level were also might be missed. Also, some farm owners and attendants were not fully motivated due to lack of incentive. Due to this some CFTT positive farms and animals were missed from collection of blood. Due to some unavoidable

circumstances collection of blood from all tuberculin tested cattle were not possible. So, blood was collected only from 52.2% of tuberculin tested (CFTT) cattle.

CONCLUSION

The overall seroprevalence of bTB (*Mycobacterium bovis*) was 7.5% in intensively managed commercial dairy farm with high yielding cross breeds. In farm level, identified risk factors by multivariate logistic regression modeling were population size and stocking density. In individual cattle level, identified risk factor was source of animal. The overall high bTB seroprevalence suggest that the percentage of late-stage diseased animals were high in dairy farms of Chattogram region and it was due to absent of bTB control measure. Our study recommends that, face in housing system has more susceptible to bTB transmission thereby we should try to avoid this system and can use face out system where there is less chance to bTB transmission among the susceptible animal. Moreover, high population size in farm has more susceptibility to bTB transmission that lower population size of the farm, so farms with a higher population should managed with extra precaution. Animal should purchase after determined whether the source stock is bTB free. Unknown source of animal should avoid during purchase as they may have bTB and some others infection in the animal. The education and awareness level for bTB among the farmers should increase which may help to prevent bTB transmission between the animal and human as well.

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Investigation of the prevalence of digestive system parasites in chickens in the Kırıkkale region

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ABSTRACT

Objective: In this study, it was aimed to investigate the prevalence of digestive system parasites in backyard chickens in the Kırıkkale region.

Material-Method: One hundred (100) faecal samples were taken by visiting the poultry houses where domestic chicken breeding was carried out. Care was taken to ensure that the faecal samples were fresh and not in contact with soil. Each faecal sample was separately placed in plastic containers with lids and delivered to Kırıkkale University, Faculty of Veterinary Medicine, Department of Parasitology, Routine and Epidemiology laboratory under appropriate conditions. The samples were analysed on the same day by native-Lugol and Fülleborn flotation technique and the faecal samples which were positive for Eimeria oocyst were sporulated in potassium dichromate for species identification.

Result: Sixty-three of the faecal samples (63%) were found to be infected with one or more parasite eggs/oocysts. Eggs/oocysts of one, two and three different parasite species were detected in 42.9%, 39.7% and 11.1% of the faecal samples, respectively. *Eimeria* spp. 13%, *Ascaridia* spp. 6%, *Capillaria* spp. 12%, *Eimeria* spp.+*Trichostrongylus tenuis* 3%, *Eimeria* spp. + *Ascaridia* spp. 3%, *Ascaridia* spp. + *Capillaria* spp. 11%, *Ascaridia* spp. + *Capillaria* spp. + *Eimeria* spp. 3%, *Capillaria* spp + *Eimeria* spp. 4%, *Capillaria* spp + *T. tenuis* 1%, *Eimeria* spp. + *Ascaridia* spp. + *Heterakis* spp. 1%, *Ascaridia* spp. + *Capillaria* spp. + *T. tenuis* 1%, *Capillaria* spp. + *Ascaridia* spp. + *Heterakis* spp. 2%, *Ascaridia* spp. + *Heterakis* spp. 2% and *T. tenuis* 1% were detected in this study. *Eimeria* spp. oocysts were morphologically identified as *E. tenella, E. necatrix, E. brunetti, E. mitis* and *E. maxima*.

Conclusion: As a result, it is thought that the parasite rate is high due to the fact that the sampled chickens are free-ranging in the natural environment, parasites are more common during the infective periods of parasites or parasite control and treatment are not performed regularly. In order to reduce the presence of parasite infections that cause yield losses, it is recommended that the animals should have access to clean feed and water sources and regular parasitic control and treatment should be carried out.

Keywords: *Chicken, Helminth, Parasite, Protozoon, Kırıkkale*

INTRODUCTION

Chickens can be produced inexpensively due to their ability to utilise feed and high productivity such as meat and eggs, and are therefore widely used as a source of animal protein for humans both in the world and in Turkey (Tosun, 2022). In recent years, free-range chicken farming has become

increasingly widespread for reasons such as the protection of animal welfare. Free-range chicken farming contributes to the reduction of feed costs by allowing chickens to exhibit their natural behaviour, benefit from fresh air, sunlight and greenery, and feed on natural foods such as worms, insects and grass. This way of feeding causes chickens to be more exposed to the infective periods of parasites. Chickens can be infected directly by ingesting oocysts, eggs and larvae of developing parasites, or by ingesting arachnid or paratenic hosts of some parasites.

Until today, studies have been carried out to determine digestive system parasites according to faecal examination in backyard chickens. There is limited number of studies conducted about this subject in Turkey, *Echinostoma revolutum*, *Echinostoma* spp., *Raillietina* spp., *Capillaria* spp., *C. caudinflata*, *Ascaridia galli*, *Heterakis gallinarum*, *Syngamus trachea*, *Trichostrongylus tenuis*, *Davainea proglottina*, *Dispharynx nasuta*, *Choanotaenia infundibulum* among helminths (Biçek et al., 2000; Köse et al., 2009; Aydın et al., 2010; Ünlü, 2012; Denizhan and Karakuş, 2019), and *Eimeria* spp. oocysts among protozoa were detected (Orunç and Biçek, 2009).

In this study, it was aimed to investigate the prevalence of digestive system parasites in backyard chickens in the Kırıkkale region.

MATERIALS and METHODS

Permission for sample collection and the conduct of the study was obtained from Kırıkkale University Experimental Animals Local Ethics Committee (Letter dated 12.12.2022 and numbered E-137009). A total of 100 faecal samples were collected from chicken coops in Kırıkkale Centre, Keskin, Balışeyh, Bahşılı and Yahşihan districts. Before the faecal samples were collected, chickens were randomly selected from the animals in different poultry houses and each of them was individually placed in separate closed cages and faecal samples were collected from the relevant places the same day. Each of the collected fresh faecal samples was placed in plastic containers with lids and transported to the laboratory under suitable conditions. The faeces were analysed on the same day by native-lugol and Fülleborn saturated saline flotation technique. The faeces found to be positive for *Eimeria* spp. were placed in Petri dishes containing potassium dichromate for sporulation of oocysts and checked daily for 7 days to determine the sporulated oocysts and species identification was made from the relevant literature (Kumar et al., 2015).

Statistical Analysis

Samples were analysed with frequency tables. The rates of detected parasites were evaluated as percentages.

RESULTS

It was found that 63% of the examined chicken faeces were infected with eggs/oocysts of at least one parasite. Among the protozoans, *Eimeria* spp. oocysts and among the helminths, *Capillaria* spp., *Ascaridia* spp., *Heterakis* spp. and *Trichostrongylus tenuis* eggs were found (Figure 1).

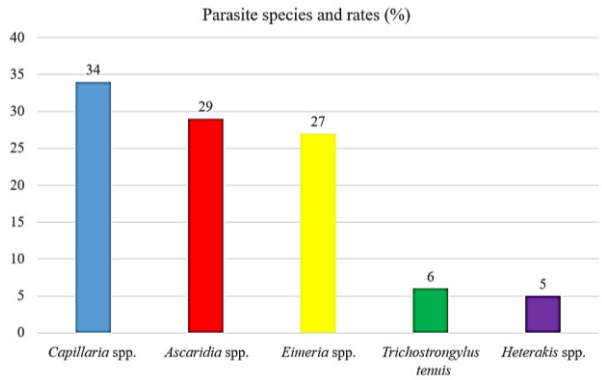

Figure 1. The ratio of parasites detected at genus and/or species level

In 42.9%, 39.7%, 11.1% and 11.1% of the faecal samples found to be positive for parasites, one, two and three different parasite species were found at the same time. The parasite eggs/oocysts and their ratios detected in the study are given in Table 1.

When the total rates of single and mixed infected faeces were analysed, the most common species was *Capillaria* spp. (34%). This was followed by *Ascaridia* spp. (29%), *Eimeria* spp. (27%), *T. tenuis* (6%) and *Heterakis* spp. (5%) (Figure 1).

Only protozoan oocysts were found in 20.6%, only helminth eggs were found in 57.2%, and eggs and oocysts of helminth+protozoan parasites were found in 22.2% of the faecal samples which were positive for parasites. All of the detected helminths belonged to nematode parasites and no trematode or cestode eggs were found.

Eimeria spp. oocysts were detected morphologically as *E. tenella, E. necatrix, E. brunetti, E. mitis* and *E. maxima* oocysts as a result of sporulation of faecal samples which were positive for *Eimeria* spp. oocysts (Figure 2).

Table 1. Parasite species and rates detected in chickens according to fecal examination

Parasite species	Number of positive samples (n)	The proportion of positive samples (%)	The proportion of total samples (%)
Eimeria spp.	13	20.6	13
Ascaridia spp.	6	9.5	6
Capillaria spp.	12	19	12
Trichostrongylus tenuis	1	1.6	1
Eimeria spp.+*T. tenuis*	3	4.8	3
Eimeria spp.+ *Ascaridia* spp.	3	4.8	3
Ascaridia spp.,+ *Capillaria* spp.	11	17.4	11
Capillaria spp.+*Eimeria* spp.	4	6.3	4
Capillaria spp. + *T. tenuis*	1	1.6	1
Ascaridia spp.+*Heterakis* spp.	2	3.2	2
Eimeria spp.+ *Ascaridia* spp. + *Heterakis* spp.	1	1.6	1
Ascaridia spp.+ *Capillaria* spp.+*Eimeria* spp.	3	4.8	3
Ascaridia spp.,+ *Capillaria* spp.,+ *T. tenuis*	1	1.6	1
Capillaria spp.+ *Ascaridia* spp. + *Heterakis* spp.	2	3.2	2

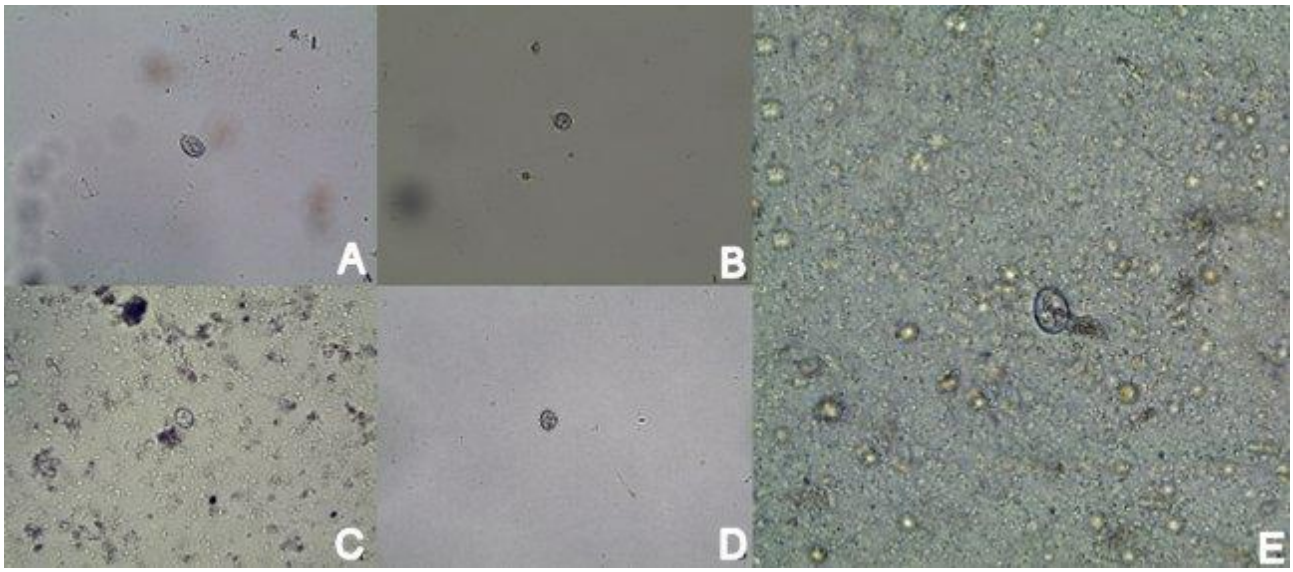

Figure 2. Sporulated *Eimeria* oocyst. **A:** *Eimeria maxima* (x10), **B:** *E. mitis* (x20), **C:** *E. necatrix* (x20), **D:** *E. tenella* (x10), **E:** *E. brunetti* (x40)

DISCUSSION

Parasitic diseases, which cause significant economic losses in the poultry sector, are usually subclinical and therefore the diagnosis of these diseases is generally neglected. In Turkey, studies have been carried out to determine helminths according to faecal examination in backyard chickens, but there are very few studies on the presence of both helminths and protozoa in general. In a study conducted in Van, the rate of endoparasites in chickens according to faecal examination was determined as 85% (Orunç and Biçek, 2009), while this rate was determined as 63% in our study. In studies conducted in the world, this rate was determined as 92.2% in the Philippines (Ybanez et al., 2018), 62.6% in Brazil (Silva et al., 2022), 60.5% in Myanmar (Win et al., 2020), 71.3% in Nigeria (Nnadi and George, 2010). The results of our study are in accordance with the results of studies in Brazil and Myanmar.

Ascaridia galli has been detected in chickens in many studies in Turkey and around the world. In studies conducted in Turkey, *A. galli* was detected in 0.21-16% of chickens according to faecal examination (Biçek et al., 2000; Köse et al., 2009; Orunç and Biçek, 2009; Aydın et al., 2010; Ünlü, 2012; Denizhan and Karakuş, 2019), whereas in studies conducted in the world, *A. galli* was detected at a rate of 12.5% in Pakistan (Sial et al., 2015), 7-17.2% in Nigeria

(Nnadi and George, 2010; Afolabi et al., 2016), 0.63-37.1% in Ethiopia (Berhe et al., 2019; Wondimu et al., 2019), 41.2% in the Philippines (Ybanez et al., 2018), 33% in Iran (Nabinejad and Noaman, 2019), and 19.16% in India (Bhat et al., 2014). In this study, *Ascaridia* spp. was detected at a rate of 6% alone and 29% in total. Considering the total rate, *Ascaridia* spp. eggs were found at a higher rate than in other studies in our country. The difference might be due to the different number of samples examined, the methods used, the feeding conditions of the animals or the climatic characteristics of the regions and therefore the ecology.

Heterakis gallinarum is a common parasite in chickens in Turkey. In the studies carried out in Turkey, the egg production of this parasite was 10.32-23.91% (Biçek et al., 2000; Köse et al., 2009; Orunç and Biçek, 2009; Aydın et al., 2010; Ünlü, 2012; Denizhan and Karakuş, 2019). In studies conducted around the world, *Heterakis* spp. eggs were found in chickens at a rate of 9.5% in India (Bhat et al., 2014), 10.4-65.6% in Ethiopia (Berhe et al., 2019; Wondimu et al., 2019), 1.8-12.6% in Nigeria (Jegede et al., 2015; Afolabi et al., 2016; Gimba et al., 2019), 59.3% in the Philippines (Ybanez et al., 2018), 18% in Iran (Nabinejad and Noaman, 2019). In the present study, *Heterakis* spp. eggs were found at a rate of 5%. Eggs of this species were not detected alone in any sample but were detected mixed with other parasites. In our study, *Heterakis* spp. detected according to faecal examination was lower than the studies conducted both in Turkey and in the world. The reason for this may be due to various conditions such as the number of samples examined, the methods used in diagnosis, and the different structures of the poultry houses where the animals were housed.

According to faecal examination in chickens, *Capillaria* species were found in 59.3% of eggs in Ethiopia (Berhe et al., 2019), 0.9-5.7% in Nigeria (Nnadi and George, 2010; Jedege et al., 2015), 49.6% in Myanmar (Win et al., 2019), 10.7% in the Philippines (Ybanez et al., 2018), 7.25% in Iran (Nabinejad and Noaman, 2019), and 3.5% in India (Bhat et al., 2014). In Turkey, the rates were 12.5-30.0% in Van (Biçek et al., 2000; Orunç and Biçek, 2009; Denizhan and Karakuş, 2019), 18% in Afyonkarahisar (Köse et al., 2009), 11.3% in Aydın (Ünlü, 2012) and 21.38% in Hakkari (Aydın et al., 2010). In our study, the rate of the causative agent was 12% alone and 34% in total. The present findings are in agreement with other studies in Turkey. When compared with the studies carried out in the world, it is higher than those detected in Nigeria and the Philippines and lower than the studies carried out in Ethiopia and Myanmar. It is thought that the reason why the rate is different from these studies is due to the fact that the care and feeding conditions and production methods of the animals are different from each other. While all of the animals sampled in our study were backyard chickens, considering the studies conducted in the world, the animals from which faecal samples were taken were obtained from free-range chickens and/or commercial enterprises.

In this study, *T. tenuis* eggs were found at a rate of 6%. In other studies, conducted in Turkey, this rate was found to be 5% in Afyonkarahisar (Köse et al., 2009); 2-7.4% in Van (Biçek et al., 2000; Orunç and Biçek, 2009; Denizhan and Karakuş, 2019), 11.9% in Hakkari (Aydın et al., 2010). In studies conducted around the world, it was determined as 3.5% in Iran (Nabinejad and Noaman, 2019), 1.6% in Nigeria (Afolabi et al., 2016), 1.72-2.5% in India (Bhat et al., 2014; Kumar et al., 2015). When the studies conducted both in various countries in the world and in Turkey are examined, it is noteworthy that *T. tenuis* is less common in chickens compared to other nematodes, similar to the results of our study.

In this study, eggs of nematodes were found among helminths, while eggs of cestodes and trematodes were not found. While cestodes and trematodes usually complete their development by using a host, nematodes usually develop without using a host. The reason why cestode and trematode eggs were not found in our study was thought to be due to the inability of chickens to reach the arachnids of these parasites due to their feeding and production characteristics.

Oocysts of *Eimeria* species causing coccidiosis, which is considered to be the most important protozoal infection in poultry, were found in 27% of chickens in this study. This rate was 31.8% in Tunisia (Kaboudi et al., 2016); 7.7-39.5% in Nigeria (Jedege et al., 2015; Afolabi et al., 2016); 5.33-81.03% in India (Bhat et al., 2014; Kumar et al., 2015); 47% in Pakistan (Sial et al., 2015), 87.75% in China (Huang et al., 2017), 20.5% in Myanmar (Win et al., 2020), 21.4-27.1% in Ethiopia (Temesigen et al., 2018; Wondimu et al., 2019), 43.2% in the Philippines (Ybanez et al., 2018) and 61.5-65.0% in backyard chickens in studies in Turkey (Orunç and Biçek, 2009; Aslan, 2017). While the *Eimeria* rate detected in our study is quite low compared to similar studies in Turkey, Pakistan, India and China, it is similar to the studies in other countries. It is

thought that the way chickens are raised, and feed and water hygiene are effective in the emergence of these results.

CONCLUSION

In conclusion, according to faecal examination, the rate of parasitic infection in backyard chickens in the Kırıkkale region was determined as 63%. It is thought that this rate is high because chickens are more exposed to the infective periods of parasites due to the free movement of chickens outside, lack of regular parasite control and treatment, and non-compliance with prevention and control measures. In order to reduce the amount of parasite infection in chickens, it is recommended that quarantine measures should be followed when new animals are taken into the poultry houses, attention should be paid to feed and water hygiene and regular parasite control and treatment should be applied.

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Investigation of the morphologic and scanned electron microscopic properties of wild boar hairs in the Balıkesir region

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ABSTRACT

Objective: Determination of species from animal hair is an effective method in veterinary forensic investigations, research, endangered species and prevention of poaching. Since the hairs are resistant to deterioration, they can be stored as evidence for many years. In addition, pig hairs are often used in making brushes. When these brushes are used in the food industry, it raises questions about halal food. This study aimed to identify these hairs by examining the hair structure of wild pigs living in the Balıkesir region and revealing their characteristics.

Materials and Methods: The hairs of 3 wild boars obtained from the İvrindi (Balıkesir) region were used. After the hairs taken from different parts of the pigs were cleaned, stereomicroscopy and macroscopic examination were performed and routine procedures were applied for scanning electron microscopic imaging.

Result: In stereomicroscopy and macroscopic examination, it was determined that the length and thickness of the hairs in different regions varied significantly. In the study, the hairs were generally bifurcated from the upper 1/3 part. In the scanning electron microscopic images, the hardened cuticle patterns on the hair shaft, which have a scaly appearance, were detected, and their measurements were made. Scanning electron microscopic images determined that there were very small bifurcations from the hair shaft. However, it was thought that these hairs could not be used for species separation, since these parts would break off in the hairs used as brushes. Significant images could not be obtained in cross-sections.

Conclusion: It is thought that it will be used as a source for the identification of the hairs of wild boars in the Balıkesir region.

Keywords: *Hair, Pig, SEM, Stereo microscope*

INTRODUCTION

Hair serves many functions, including thermoregulation and protection. It maintains temperature in animals by retaining heat or preventing cold. The hair can also provide camouflage and act as sexual attraction (Breehl and Caban, 2022; Grubbs et al., 2022). The hair follicle also has a wide variety of functions, including thermoregulation, physical and immunological protection against external aggressions, sensory perception, social interactions (Welle and Wiener, 2016). There are many factors that affect the hair structure. Hormones, vitamins, glandular secretions, environment, genetics, nutrition and trauma can alter the normal state of hair growth. Thyroid hormones physiologically stimulate hair growth. Adrenocorticotropic hormone deficiency,

disease, injury, environmental factors and stress also have a negative effect on hair growth (Choudhary et al., 2012).

Analysis of animal hair can be used in veterinary forensic research and biology as an effective tool to deter the illegal trade, slaughter and poaching of animals, including endangered species. It can be taken as physical evidence, as the hairs show strong resistance to degradation and rot. Mammalian hairs can be easily collected, preserved and transported to the laboratory for species identification by microscopy (Nowak, 1998). The hair sample can be examined by direct observation of all hairs using a light microscope (Valente, 1983; Oli, 1993; Wallis, 1993; Taru and Backwell, 2013) where the scanning electron microscope provides the advantage of higher magnification (Andy and Tillman, 2006; Aris and George, 2008; Dehury et al, 2019).

Scanning electron microscopy can be used in wildlife forensics for species identification (Short, 1978). The surface pattern, cross-section, and medullary index provide information of the specimen that can be used as geographic region and species identification tool. The cuticle is a useful tool for distinguishing species between wild ungulates and for deer to separate juveniles from adults and winter from summer fur (Meyer et al., 2001).

Electron microscopic studies of mammalian hair have also been done with transmission electron microscopy (Muto et al., 1981; Slepecky et al., 1981; Weedon and Strutton, 1981; Maxwell et al., 1982) and scanning electron microscopy (Short, 1978; Riggott and Wyatt, 1980). Combinations of both methods have also been used (Hino et al., 1982; Raphael et al., 1982). Cross and longitudinal sections of hairs or sanded hairs were examined by scanning electron microscopic imaging (Hess et al., 1985).

Pigmentation and hair size are anatomical structures affected by different variables, such as seasonality and association with growth, so their diagnostic utility is limited. Pigmentation and hair size vary with age, season and body area. Also, under the influence of digestive enzymes, pigmentation can deteriorate, while hair size can be changed, for example, due to fragmentation. These are factors that make diagnosis difficult. Therefore, scanning electron microscopic examination is very important (Kennedy and Carbyn, 1981; Amerasinghe, 1983).

In a study, surface scale patterns, transverse and longitudinal sections and sanded feathers of animals belonging to the families Tayassuidae and Suidae were examined by scanning electron microscopy. When the cross-sectional faces of Tayassu and Catagonus hairs were examined, cortical layers of varying thickness, protrusions arranged according to certain intervals and heights, and spongy areas were encountered, showing slight differences between genera and species (Hess et al., 1985).

Hairs are used to make some brushes. When these brushes are used in the food industry, it raises questions about halal food.

This study was carried out to examine the hair structure of wild pigs living in the Balıkesir region, to reveal their characteristics and to facilitate their diagnosis.

MATERIALS and METHODS

The study was carried out using the hairs of 3 wild boars (Sus scrofa) in İvrindi region (Balıkesir) in the spring season. No other wild pig breeds were found in Balıkesir region. The hairs taken from different parts of the pigs (inguinal, caudal and lumbar regions) were cleaned with detergent and rinsed with distilled water, and stereomicroscopy and macroscopic examination were performed, routine procedures were applied for scanning electron microscopic imaging.

For scanning electron microscopy studies, the hair samples were cut into 5 mm pieces, 3 mm from the root, and placed on the sample holder (on the adhesive tape) and prepared by gold plating for 20 seconds at 5 millibar vacuum and 5 mA current. The samples were examined under a scanning electron microscope. The hair pattern inter-scale distance for each animal species was measured using the SEM software digital scale.

For this study, research permission was obtained from the Ministry of Agriculture and Forestry with the number E-21264211-288.04-9829863 (10.05.2023).

RESULTS

It is important to identify the animal species from the hairs. Macroscopic analysis of hairs represents only the first step in the process of identifying an unknown hair sample. Among wild mammals, macroscopic hair species identification can only be definitively defined in wild boar hairs without the

aid of microscopic analysis. The boar's hairs are easily recognizable to the naked eye by the general appearance of its hairs, which have been bifurcated at least once. In piglets, bifurcation does not appear.

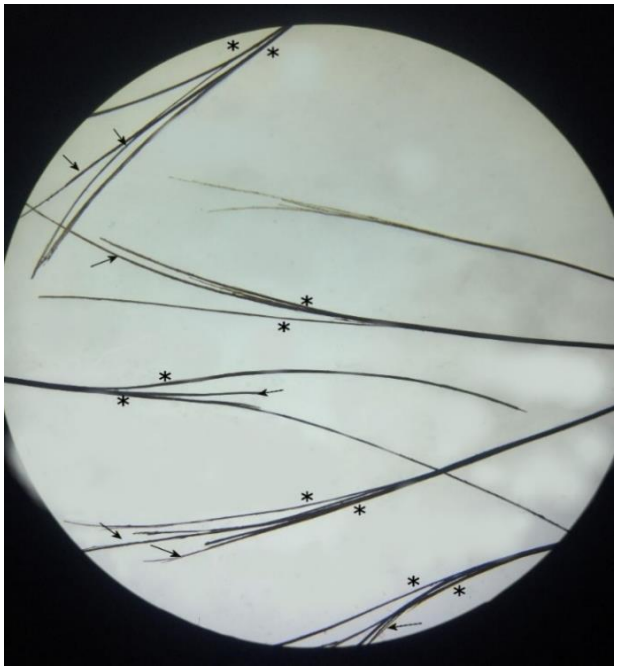

Figure 1. Stereo Microscope image, Asterisk: primary branches; arrow: secondary branches.

On a white background, bifurcations were clearly observed in the macroscopic examination of the hairs. These bifurcations could be from the same level as well as from different levels. In the study, it was observed that the hairs were generally bifurcated from the upper 1/3 part. This first bifurcation was called the primary bifurcatio, and the hair originating from this region was called the primary hair (Figure 1).

The number of primary bifurcations ranged from 2 to 9. This number was found to be less in fine hairs. Hairs that did not show bifurcation were also observed. It was observed that there were re-bifurcations from these bifurcated fragments in some large hairs. This is called secondary bifurcation, and the hair originating from this region is called secondary hair (Figure 1).

It was determined that the hairs separated from the primary bifurcatio were divided into two or more parts. It was observed that these bifurcations were directed towards the tip of the hair, but bifurcations directed in the opposite direction were also detected (Figure 2). It was observed that the hairs especially in the caudal and dorsal region were quite thick.

Table 1. Macroscopic features of the hair of wild boars.

Hair pattern position	Surface condition	Distance between consecutive patterns	Pattern	Appearance
Transverse	Smooth (Except secondary bifurcatios)	Narrow	regular wave	Bright

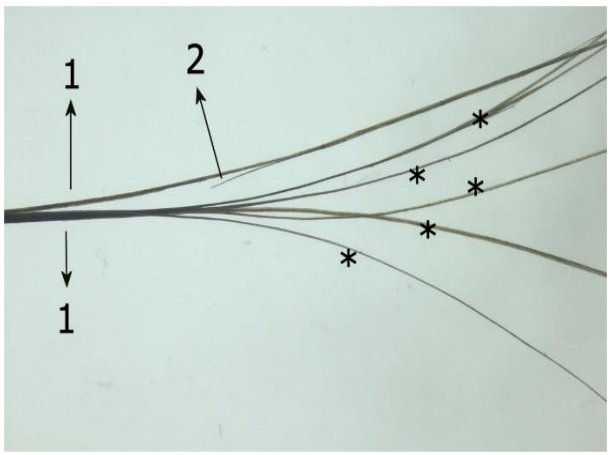

Figure 2. Stereomicroscopic hair image, 1- Primary branch, 2- A posteriorly directed secondary branch, Asterisk: secondary branch.

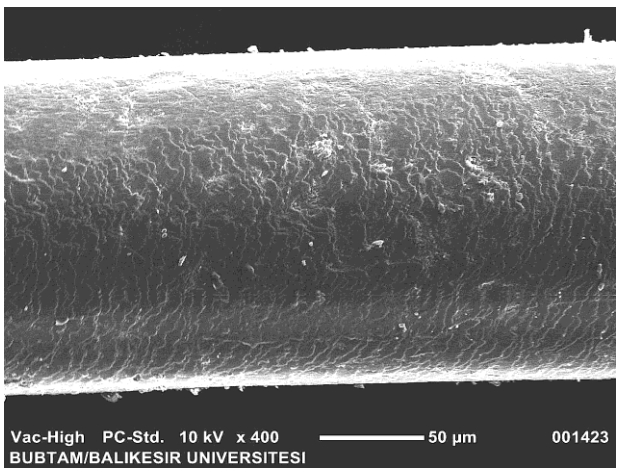

Figure 3. Hair surface, scanning electron microscopic image.

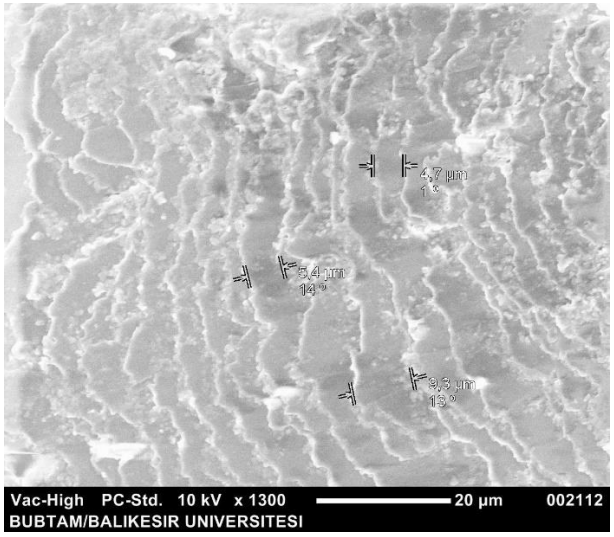

Figure 4. Hair surface, scanning electron microscopic image

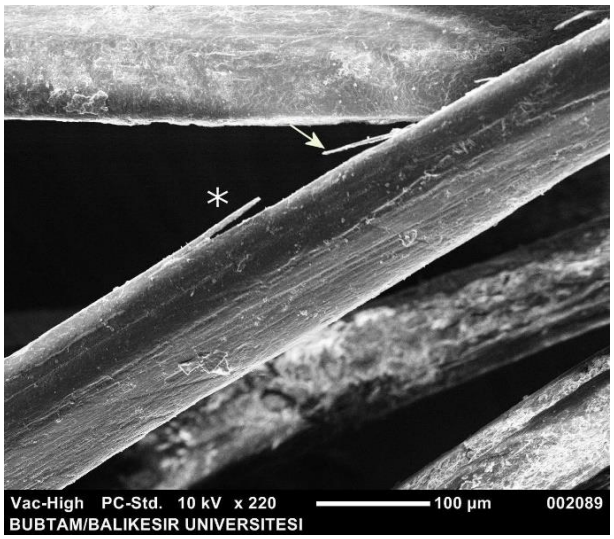

Figure 5. Asterisk: secondary hair extending towards the hair tip, Arrow: Secondary hair extending towards the hair root -in the opposite direction.

The pattern position, surface condition, and distance between successive patterns of the hairs taken in the study were examined. In the pattern position, the direction of the pattern was determined. In our study, it was determined that the pattern direction was transverse, and when the surface condition was examined, it was generally a smooth surface. However, considering the primary bifurcations separated from the hair body and the secondary bifurcations separated from them, these parts were not considered as smooth surfaces.

The distance between successive patterns was measured in electron microscopic images. This range is set very narrow. The pattern is determined as regular wavy. Overlapping, regular wave patterns were observed on the edges of the flakes on all surfaces examined. The general appearance was determined as bright (Table 1).

In stereomicroscopy and macroscopic examination, it was determined that the length and thickness of the hairs in different regions varied significantly. It was observed that the hairs especially in the caudal and lumbar regions were quite thick. No difference in pattern was observed.

In the scanning electron microscopic images, the hardened cuticle patterns in the hair shaft, which have a scaly appearance, were detected and their measurements were made (Figure 3-4.). It was also determined that there are very small bifurcations from the hair body (Figure 5). In the cross-sections, a gap was observed only in the body section.

DISCUSSION

Scaly patterns of plumage of domestic pig (Sus scrofa domesticus), European wild boar (Sus scrofa scrofa), and Warty pig (Phacochoerus aethiopicus) have been reported. It has been noted that it seems impractical to try to distinguish species and subspecies based solely on scale patterns (Wilford et al., 1985). With the pattern determined in our study, it was determined that the patterns of Domestic pig (Sus scrofa domesticus), European wild boar (Sus scrofa scrofa) and Warty pig (Phacochoerus aethiopicus) were basically the same.

The plumage of the three subspecies of Tayassu pecari has been reported to be relatively straight, short and flattened, with dark hair colors and frayed tips of most (Hess et al., 1985). In addition, the fact that the hair is the same color from one end to the other is consistent with the findings of our study. In this respect, the hairs in the study are similar to tayassu pecari. However, in our study, it was observed that the ends of most hairs were not frayed.

The feathers of the Tayassu tajacu are relatively long, not as straight as those of the Tayassu pecari, and color variations are noted from one end to the other. It has also been reported that the feathers exhibit characteristic fluctuations, some of which are not frayed at the ends, and are variable in length and diameter (Hess et al., 1985). In the presented study, it was determined that all hairs were relatively short, straight, unwavering and without color differences from one end to the other.

In a study comparing the summer plumage of C. Wagneri with the winter plumage, it was reported

that the winter plumage is larger and longer, but the morphological features are otherwise the same (Hess et al., 1985). Since the study examined the hairs in the first spring season, such a comparison could not be made.

According to Anna Maria De Marinis and Alessandro Asprea, fawns and wild cattle can be distinguished from adults by having an irregularly wavy cuticular pattern along the length of their feathers. It has been reported that all hair samples of young animals examined show this feature from birth to 3-4 months of age (Jedrzejewski et al., 1992). During the first molt, it has been observed that the offspring transform their natal fur to a fur similar to that of the adults (Ryder, 1960; Johnson and Hornby, 1980; Jedrzejewski et al., 1992). Since adult animals were used in our study, no comparison could be made.

Although it has been reported that holes made by ectoparasites are evident in the hair surface images (Hess et al., 1985), these holes caused by ectoparasites were not observed.

Adults and offspring of sheep have been reported to show wavy and dull hair (De Marinis and Alessandro, 2006). In our study, no wavy hair was found and the hairs were brightly colored. Also, no fragmented hairs like those found in carnivores were found.

Worn, bifurcated guard hairs characterize domestic and wild boars and crossbreeds (Marchinton et al., 1974; Hess et al., 1985; Mayer and Brisbin, 1991). Boar subspecies and other Suidae species typically have hairs with frayed ends (Koppiker and Sabnis, 1977; Amerasinghe, 1983; Hess et al., 1985). In addition, Tayassuidae species have bifurcated hairs at the tips (Hess et al., 1985). The degree of wear can be correlated with the chronology of hair regeneration. The significance of this character of hair morphology is unknown (Hess et al., 1985). In our study, no significant wear was observed on the hair tips, but bifurcations were detected.

It has been determined that the cross-sectional faces of the hairs of Tayassu and Catagonus species have cortical layers of different thicknesses, protrusions arranged according to certain intervals and heights, and spongy areas that show slight differences (Hess et al., 1985). In the study, however, such regular and intermittent images could not be obtained, it was determined that there was only a gap in the middle of the hair.

Although Hess et al. (1985) stated in their study that parasites or their effects were observed in the hair, ectoparasites and their effects were not observed in our study.

The location of the scales relative to the longitudinal axis (transverse- middle) of the hair, the structure of the free edges of the scales (straight - wavy), the distance between the scales edges (distant - near), and the scaled pattern (regular mosaic, regular and irregular wave, Ω-shaped) diagnosis of the hair (De Marinis and Alessandro, 2006). In our study, it was determined that the pattern direction of the hair is transverse, the distance between the hair flake edges is narrow, the structure of the flake free edges is regularly wavy as a scale model, and the flakes are overlapped.

CONCLUSION

In the study, the hairs of wild boars living in the Balıkesir region were examined. Their most important bifurcation, which is used to distinguish them from other hairs macroscopically, was determined in detail. Micro bifurcations were also detected in scanning electron microscopic images, which are not encountered in the literature. The posterior ones of the bifurcations that normally tend towards the tip of the hair were also observed.

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Physiological and histopathological effects of nettle seed (Urtica pilulifera), grape seed (Vitis vinifera), flaxseed (Linum usitatissimum) in broiler

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ABSTRACT

Objective: In this study, it was aimed to investigate the effects of nettle seed, grape seed and flaxseed added to the broiler diet on body weight, electrocardiogram, hematological and histopathological parameters.

Materials and Methods: In this study 40 daily Ross 308 female broilers were used. Chicks were divided into 4 groups of 10 chicks each. During 42 days, in addition to standard broiler feed, nettle seed, grape seed and flax seed with 30 gr/kg/day were added to groups II, III and, IV respectively. *The values of* erythrocyte (RBC), leukocyte (WBC), platelet (PLT), hematocrit (HCT), hemoglobin (Hb) and percentages of white blood cells were determined by conventional methods. The liver, kidney, spleen and lung tissues held in formaldehyde (10%), were passed through the alcohol and xylose serial in routine tissue tracking and were buried in paraffin blocks. They were prepared on the lam and stained with Hematoxylin-eosin (HE) and examined by light microscopy.

Results: It was determined that body weight was higher in the control group than those in the other groups in the second weighing and there was a decrease in the number of heart beat in group IV ($p \leq 0.05$). There was no significant difference with regard to hematological and histopathological findings.

Conclusion: Even if the additional nettle seed, grape seed and flaxseed (30 mg/kg) to broiler rations did not have any positive effect on body weight gain, it is also important that it has no negative effect on blood, heart, liver, kidney, lung and spleen.

Keywords: *Body weight, Electrocardiogram, Blood parameters, Tissue examination, Broiler, Nettle seeds, Grape seeds, Flax seed*

INTRODUCTION

Nowadays, it is very important to supply the increased food need, and animal foods are becoming a necessity to breed healthy generations and diets. Chicken meat, starting to get ranked first among animal foods, come at the beginning of food that people need for adequate and proper nutrition. There are some studies in which the effects of different plants were searched in order to maintain body weight gain healthily without changing the animal genetics and disordering its health (Brenes et al., 2010).

Many antibiotic alternatives with growth-promoting effects, such as herbs, are of interest to researchers (Omerovic et al., 2016). Stinging Nettle is one of the medicinal plants that has recently attracted attention as a phytogenic feed additive in poultry diets and has a long history of traditional medicinal use in many countries. (Ahmed and Parsuraman, 2016).

The cardiovascular effects of dietary flaxseed include an antihypertensive effect, an anti-atherogenic effect, a decrease in cholesterol, an anti-inflammatory effect, and suppression of arrhythmia. Although flaxseed is not very well known, it also consists of other potential biologically active compounds, such as proteins capable of exerting a biological effect, cyclinopeptides and cyanogenic glycosides. These compounds may also be responsible for the cardiovascular effects of flaxseed (Parikh et al., 2018).

Flaxseed and oil, nettle, grape seed and pomace have been used among plant-derived feed additives. Some researchers utilized flax seed and oil as feed additives to be able to maintain cardiovascular health by enriching poultry meat with omega-3 fatty acids (Chanmugam et al., 1992). Although poultry meat is preferable because of containing less fat, it can cause malnutrition in terms of polyunsaturated fatty acids (omega-3). Omega-3 fatty acids are effective in the prevention of coronary heart disease, cancer, arteriosclerosis and diabetes in people. Therefore, the number of studies, directed toward the enrichment of poultry meat with omega-3 fatty acids for adequate and proper nutrition of humans have increased.

Flaxseed is a rich source of α-linolenic acid (18:3 n-3). Feeding broilers flaxseed can increase n-3 fatty acids in meat tissues. However, non-starch polysaccharides in flax seed decrease nutrient digestibility and can have a negative impact on bird performance and muscle fatty acid content. The addition of carbohydrase enzymes to flax-based broiler diets can decrease the anti-nutritive effects of non-starch polysaccharides. (Apperson and Cherian, 2017).

Nettle contains formic acid, a high amount of chlorophyll, flavonoids, plant sterols, plant enzymes, phenylpropanoids, coumarins, terpenoids, potassium salts, vitamin C, polysaccharides, plant lignane, and lectin agglutinins with low molecular weight in their roots (Akbay et al., 2004). It was shown that there are many effects of polyphenols-rich grape pomace and seed, and phenolic compounds extracted from grape seed in some studies (Brenes et al., 2010).

The effects of active substances obtained from all medicinal and aromatic plants on poultry should be evaluated as a result of analyzes and commercials should be tested on animals alone or in combination, they should be added to the economy by mass production and contribute to the use of different feed additives (Kutlu ve Şahin, 2017).

Recently, the importance of some plant extracts in animal nutrition (Zajac et al., 2020; Saeed et al., 2021), hematological (Taş et al., 2011; Comba et al., 2014; Comba et al., 2016; Zajac et al., 2020; Comba et al., 2020; Abdul-Majeed, 2021; Çelik et al., 2022; Vadi and Comba, 2023) and histopathological (Comba et al., 2017; Yıldırım et al., 2017; Mis et al., 2018) studies on the effects of parameters are available in the literature. In addition, the effects of flaxseed 0%, 2.5%, 5.0%, 7.5% and 10% on growth performance in broiler chickens were investigated (Mridula et al., 2015). But, no studies were encountered in which the effects of giving the same amount (30 mg/kg) of nettle seeds, grape seed and flax seed in addition to broiler ration on body weight, electrocardiograms (ECG), some hematological and histopathological parameters were researched. In this study, it was aimed to investigate the effects of additional doses of nettle, grape and flax seed with 30 mg/kg which are used to improve *feed* efficiency and performance in poultry farming to the standard broiler ration on body weight, ECG, hematological and histopathological parameters.

MATERIALS and METHODS

Study Groups

This study was carried out with the decision of the Atatürk University Faculty of Veterinary Medicine Unit of Ethics Committee (2018/41). A total of 40 Ross 308 female broiler chicks were used in this study. Standard temperature (23±2 °C), humidity (50%±10) and light (23/1 h light/dark) were applied by providing appropriate conditions in the stell cage. Chicks were divided into 4 groups of 10 chicks each as control and experimental groups. The diets and drinking water were provided ad libitum. Additionally, nettle seed (30gr/kg/day), grape seed (30gr/kg/day) and flax seed (30gr/kg/day), were given to groups II, III and IV respectively, during 42 days, taken from local herbalists in Turkey. The samples were chopped into small parts with a blender. Compositions of all supplementary foods were determined according to AOAC (1994). The diets were isoenergetic and isonitrogenous. All the experimental diets were formulated to meet the minimum nutrient requirements of broilers (NRC, 1994). They were weighed every 10 days during the study period. After 42 days of work, Electrocardiograms (ECG) and blood, liver, kidney,

spleen and lung tissues were taken for all groups. The tissues were taken in formaldehyde (10%) and were examined histopathologically.

ECG

ECGs were taken by a device *(Cardio fax 6851, Nihon Kohen, Tokyo)* with an *electrode alligator Clip*. Electrodes were placed where *M. gastrocnemius* at the lower ends of the right and left legs and forepart of wing-body linkup of chickens are connected, after the gel was applied, in a quiet and dim environment. Animals were waited to sedate by wrapping lightweight cloth for 5 minutes. ECGs were taken at a deflection of 1mV = 10mm and a rate of 50 mm / sec. The derivations of Bipolar (I, II, III) and augmented unipolar (aVR, aVL, aVF) extremities were printed. The evaluation of duration and amplitudes of the waves in the trace was performed in the derivation of II, as calculation of the electrical axis was performed in the derivation of II and III (Figure 1).

Blood Collection

After ECGs were taken, blood samples from V. Subcutaneous of chickens were taken in an EDTA tube. The numbers of Erythrocyte (RBC), leukocyte (WBC) and platelet (PLT), hematocrit values (Hct) and the amount of hemoglobin (Hb) were estimated by hemocytometric method using Natt-Herrick solution, by micro hematocrit method and by Sahli's acid hematin method spectrophotometrically, respectively. Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular *Hemoglobin Concentration* (MCHC) were calculated with Wintrobe's formulas red cell indice. Blood smears prepared from specimens were stained with the Grunewald-Giemsa staining method and percentages of white blood cells were determined.

Tissue collection

The liver, lung, kidney and spleen tissues of chickens were held in 10% formalin solution for histopathological examinations. After the tissues were fixed in 10% formalin solution for 48 hours, they were washed in running tap water for 10 minutes. Then they were passed through alcohol (70°, 80 °, 90 °, 96 ° and 100 °) and xylol series in routine tissue tracking and they were buried in paraffin blocks. A 4-*micrometer-thick cross-section* was received from each block and it was prepared on the lam for histopathological examinations. Preparations were stained with Hematoxylin-eosin (HE) and examined by light microscopy.

Statistical Analysis

Descriptive Statistics were expressed as Average, and Standard Deviation for the features studied. Kruskal Wallis test was used to compare the groups in terms of these characteristics. The Friedman test was used to compare the times within the groups. The statistical significance level in calculations was taken as 5% and SPSS statistical package program was used for calculations.

RESULTS

The intra-group means and standard deviations of the live weights (Table 1), electrocardiographic measurements (Table 2) and hematologic values (Table 3) were given for all groups. The liver and kidney (Figure 2), lung and spleen tissues (Figure 3) were examined histopathologically.

According to Table 1, there is no significance among the groups in the 1st and 4th weighing ($p \geq 0.05$) while the control group was found to have statistically high body weight compared to the other three groups in the 3rd weighing ($p \leq 0.05$). In addition, at the 2nd weighing the body weight in the control group was found higher than in the group IV ($p \leq 0.05$).

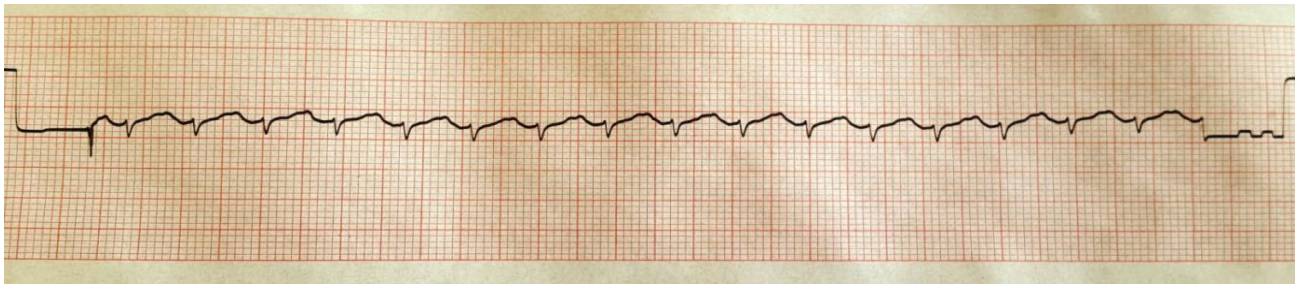

Figure 1. Electrocardiography (1mV and 50 msn) in chickens added flax seed to their feed.

Table 1. The average body weights with standard deviations for all groups (g).

Parameters	Group I (Control) X ± SD	Group II (Nettle seed) X ± SD	Group III (Grape seed) X ± SD	Group IV (Flax seed) X ± SD	p
1.weighing (1.day)	65±4.15	63±5.24	61±4.46	59±5.52	≥0.05
2.weighing (10. days)	132.10±10.24[a]	126.6±11.41[ab]	123.1±9.54[ab]	118.2±8.75[b]	≤0.05
3. weighing (20. days)	540.5±50.74[a]	448.3±46.68[b]	464.7±60.52[b]	445.3±55.46[b]	≤0.05
4. weighing (30. days)	1046.7±112.45	1011.7±128.51	1016.3±100.36	984.3±97.35	≥0.05
5.weighing (42. days)	2300.0±162.45	2240.0±155.68	2250.0±169.15	2283.5±175.23	≥0.05

a,b: The differences among groups carrying different letters in the same column are statistically significant ($p<0.05$).

Table 2. Mean and standard deviations of some ECG parameters in all groups.

	Group I (Control) X ± SD	Group II (Nettle seed) X ± SD	Group III (Grape seed) X ± SD	Group IV (Flax seed) X ± SD	p
No of heart beats per min	373±25[a]	355±25[a]	350±28[a]	310±23[b]	≤0.05
QRS wave (sec)	0.027±0.07	0.028±0.06	0.028±0.07	0.029±0.06	≥0.05
QRS wave (mV)	0.25±0.08	0.26±0.06	0.25±0.07	0.24±0.07	≥0.05
T (P+T) wave (sec)	0.048±0.014	0.049±0.013	0.049±0.013	0.051±0.013	≥0.05
T (P+T) wave (mV)	0.020±0.08	0.020±0.07	0.019±0.06	0.020±0.07	≥0.05
Q-T interval	0.13±0.018	0.13±0.017	0.12±0.016	0.14±0.017	≥0.05
Mean electrical axis of the heart	-82(-60- -120)	-90(-55- -120)	-85(-58- -115)	-84(-55- -118)	≥0.05

a,b: The differences among group averages carrying different letters in the same lines are statistically significant

Table 3. Mean and standard deviations of some hematological parameters in all groups.

Parameters	Group I (Control) X ± SD	Group II (Nettle seed) X ± SD	Group III (Grape seed) X ± SD	Group IV (Flax seed) X ± SD	p
WBC (10^3/mm^3)	14.42±2.45	15.59±2.34	13.24±1.84	15.78±1.96	≥0.05
Lymphocyte (%)	70.4±8.4	70.2±10.2	71.6±6.7	69.9±8.4	≥0.05
Monocyte (%)	5.5±1.4	5.8±1.2	5.1±2.4	6.4±2.1	≥0.05
Pseudo Eosinophil (%)	16.2±3.1	16.4±4.2	16.6±4.7	15.6±5.3	≥0.05
Eosinophil (%)	7.1±2.3	6.6±1.4	6.4±1.6	7.6±2.1	≥0.05
Basophils (%)	0.5±0.04	0.6±0.01	0.5±0.05	0.4±0.07	≥0.05
RBC (10^6/mm^3)	1.81±0.24	1.86±0.57	1.77±0.38	1.93±0.48	≥0.05
HCT (%)	19.75±1.72	19.94±2.61	18.6±1.82	20.84±2.26	≥0.05
Hb g/dl	8.42±0.53	8.06±0.74	8.12±0.92	8.66±0.71	≥0.05
MCV (fl)	108.42±2.74	107.64±3.14	109.22±2.36	110.2±3.52	≥0.05
MCH (pg)	45.64±2.45	43.72±3.64	45.04±3.52	44.76±3.26	≥0.05
MCHC (g/dl)	41.26±1.24	41.28±1.47	42.74±1.25	40.84±2.13	≥0.05
THR (10^3/mm^3)	40.25±5.61	41.12±7.35	41.3±6.53	40.02±7.42	≥0.05

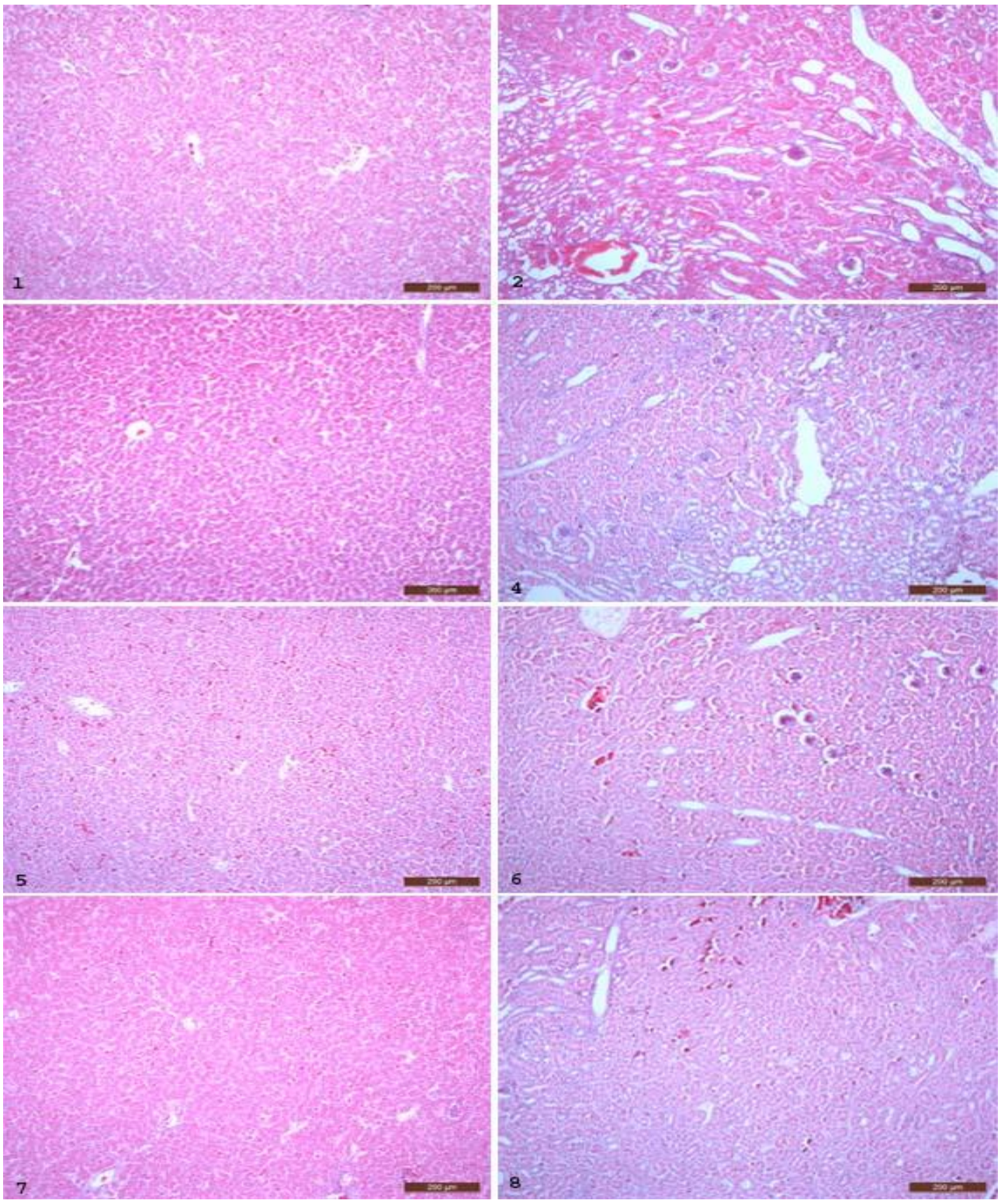

Figure 2. The normal histological structures of the liver and kidney tissues of broiler chickens in Group I (control group) (1-2), the normal histological structures of liver and kidney tissues of broiler chickens in Group II (nettle) (3-4), the normal histological structures of the liver and kidney tissues of the broiler chickens in Group III (grape seed) (5-6), the normal histological structures of the liver and kidney tissues of the broiler chickens in Group IV (flax seed) (7-8). Bar: 200 μm HxE

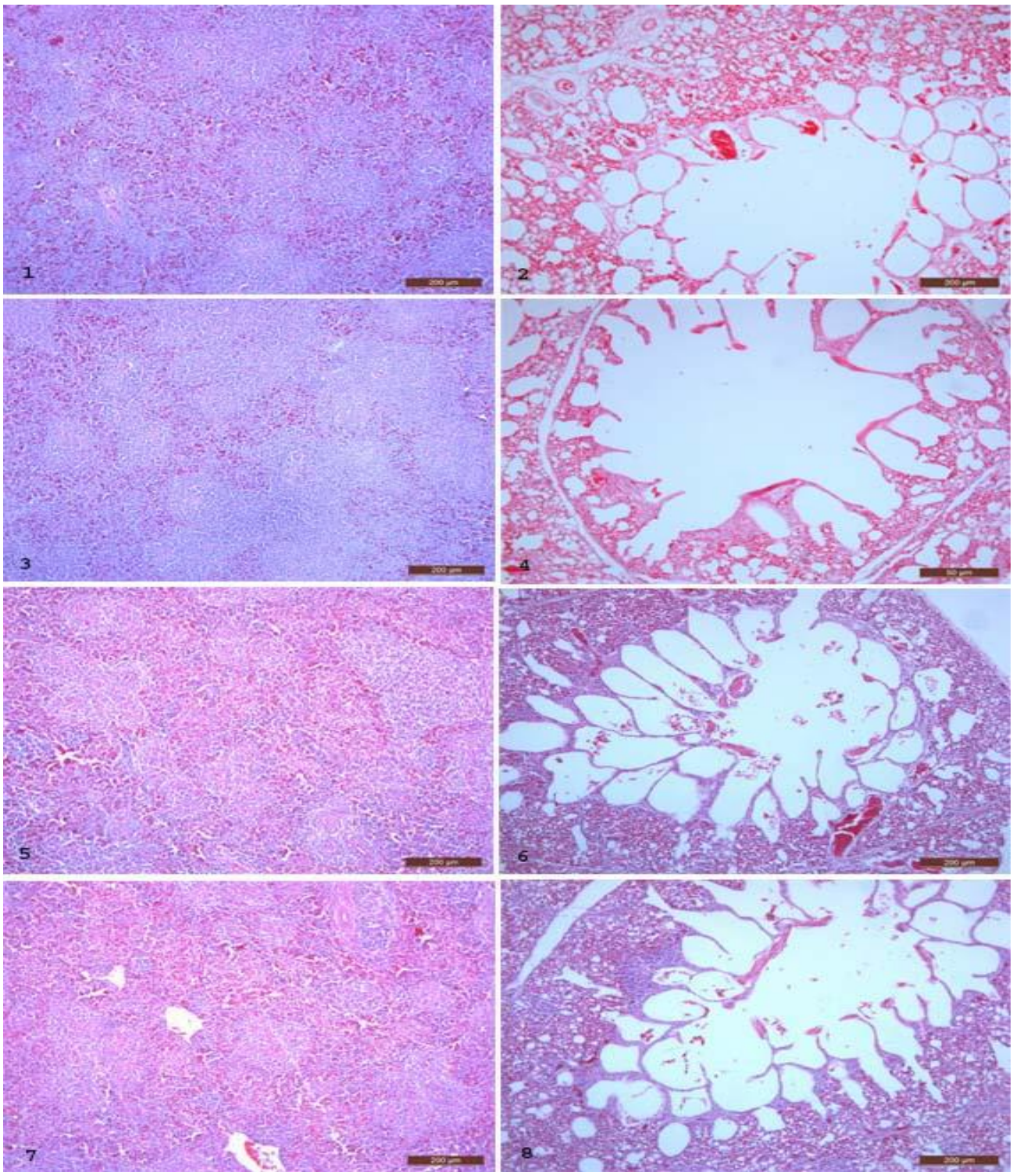

Figure 3. The normal histological structures of the spleen and lung tissues of broiler chickens in Group I (control group) (1-2), the normal histological structures of spleen and lung tissues of broiler chickens in Group II (nettle) (3-4), the normal histological structures of the spleen and lung tissues of the broiler chickens in Group III (grape seed) (5-6), the normal histological structures of the spleen and lung tissues of the broiler chickens in Group IV (flax seed) (7-8). Bar: 200 μm HxE

The number of heartbeats in group IV was lower than those in other groups according to Table 2 ($p \leq 0.05$). A significant difference cannot be found in the other ECG parameters among the groups ($p \geq 0.05$).

According to Table 3, there was no significant difference between the control and experimental groups in terms of hematological parameters.

Histopathological findings

It was not encountered with any toxicological findings as the results of necropsy and histopathological examination of liver, lung, kidney and spleen tissues. In all groups, remark cords were in normal sequence, the sinusoidal spaces were regular no pathological conditions were found in the portal and central regions in the livers (Figures 2-1, 3, 5, 7). Bronchus, bronchial, and alveoli had a normal histological appearance in the lungs in all groups (Figures 3-2, 4, 6, 8). There were not any pathological conditions in the kidney, tubules or glomerulus in all groups (Figures 2-2, 4, 6, 8). It was seen that follicular structures were protected in the spleen in all groups (Figures 3-1, 3, 5, 7). There were not any histopathologic changes among the groups with regard to histopathological evaluation.

DISCUSSION

The inclusion of nettle in the diet significantly reduced liver, heart, bursa and abdominal fat compared to the control. Ahmadipour and Khajali (2019) reported that when 1% and 1.5% nettle were included in broiler rations, body weight gain was significantly improved, but feed intake did not change significantly. In another study (Hashemi et al., 2018), in which stinging nettle leaf extract powder was added to broiler rations at 0.15%, 0.20 and 0.25% levels, it was stated that it resulted in significantly better growth. Flaxseed supplementation did not affect the weekly body weight of broiler chicks during the first three weeks, but thereafter it reduced significantly with increasing levels of flaxseed in the diets. Birds fed on 10% flaxseed showed a reduction of 10.08% in body weight as compared to the control group. Diets containing 5.0–7.5% flaxseed resulted in significantly lower weight gain, higher feed conversion ratio, energy efficiency ratio and lower protein efficiency ratio as compared to control and 2.5% flaxseed diets. (Mridula et al., 2015).

In the present study, it was determined that nettle seed, grape seed, and flax seed extracts added to the standard ration affected the body weight averages between the groups in the second and third weighing, but did not affect the first and fourth weighing. These weighing values with 10-days intervals were determined in accordance with the literature (Eser et al., 2012).

Nettle supplemented in broiler diets exerts positive effects regarding production performance. Nettle provides nutrients and bioactive components, which stimulate growth and feed utilization, modulate metabolic processes and support immune system in broilers (Milosevic et al.,2021). In a study conducted by Al-Salihi et al., (2018) with the extract of nettle leaves added to drinking water at a concentration of 10, 15 and 20 ml/l, the immunostimulatory effect of stinging nettle in broilers was reported. Hashemi et al. (2018) reported a positive effect of nettle on hemoglobin, hematocrit, and blood content in broiler chickens fed increasing amounts of nettle. Furthermore, nettle extract can stimulate the innate cell mediated immune reaction by lymphocyte propagation in laying hens (Sandru et al., 2007). In another study flaxseed (15%) treatments did not induce changes in the levels of such indices as RBC, MCHC, MCH, MCV, and PCV. In contrast, the dietary inclusion of flaxseeds to the diet decreased the hemoglobin level in comparison with the control (by 9%) and the other treatments (Zajac et al., 2020). Furthermore, adding nettles (0.25 or 0.50 %/kg diet) showed that crushed nettle plant led to an increase in the values of packed cell volume, hemoglobin, mean corpuscular hemoglobin concentration, and it's shortened clotting time, as well as a significant increase ($p \leq 0.05$) in the number of basophils compared to the control group (Abdul-Majeed at al., 2021). In our study, the change in blood parameters was not statistically significant. We think that this situation may be due to the dose difference as well as related to the way the substance is administered.

It is known that factors such as race, age, gender, pregnancy, lactation, muscle activities, region, season, ambient temperature, care and nutrition have an effect on hematologic parameters, ECG values, growth and development in animals (Belge et al., 2003; Çınar et al. 2006). The taste, flavor and consumption amount of the feed additives are among the significant factors affecting the quality of feed utilization in poultry feeding (Çınar and Dönmez, 2001). It has also been reported that polyphenols (flavonoid, anthocyanin, elagi-tannin, proanthocyanidin) can adversely affect the performance of animals by reducing the absorption of fat and proteins by composing complexes with macromolecules (such as fat and protein) and enzymes (protease and lipase) in the digestive tract (Manach et al., 2004).

It was declared that the addition of 0.6, 1.8 and 3.6 % of grape pomace extracts (Brenes et al., 2010), and 0.5, 1 and 2 % of milled grape seeds or 200, 400 and 800 ppm grape seed extracts (Turan ve Öztürk,,

2010) to broiler feed did not affect body weight, feed consumption or the rate of feed utilization. Hughes et al. (2005) reported that the addition of 0.2, 0.5 and 1.0% of grape seeds to broiler feed did not have a negative effect on feed intake and body weight but the addition of 3.0% of that reduced these parameters cumulatively. Brenes et al., (2010) stated that high levels of consumption of polyphenols in the grape seed can cause adverse effects on animal health and performance. Our results have supported the literature in terms of there were no differences for in ECG, blood and histopathological parameters between the control and added grape seed group (Hughes et al., 2005; Turan and Özturk, 2010; Brenes et al., 2010).

Cardiovascular disease remains the leading cause of mortality and morbidity worldwide. The inclusion of functional foods and natural health products in the diet are gaining increasing recognition as integral components of lifestyle changes in the fight against cardiovascular disease. Several preclinical and clinical studies have shown the beneficial cardiovascular effects of dietary supplementation with flaxseed (Parikh et al., 2018).

Dietary flaxseed protects against ventricular fibrillation induced by ischemia-reperfusion in normal and hypercholesterolemic Rabbits. (Ander BP et al., 2004). It was reported that the addition flaxseed oil at different levels to broiler ration did not affect feed consumption, body weight gain, and the feed utilization ratio (Manilla et al., 1999; Çebi, 2010). However, Kralik et al. (2003) detected that the addition of 13.5% of flax seed to broiler increased the levels of mono-unsaturated fatty acids and α-linoleic fatty acids in breast meat and abdominal fat while it decreased the level of saturated fatty acids significantly. Furthermore, they expressed that the ratio of omega-6/omega-3 in breast meat and abdominal fat decreased significantly. Similarly, Lopez-Ferrer et al. (2001) detected that the addition of flax seed oil reduced the amount of saturated fatty acids. Reducing blood LDL significantly, makes using of flax seed oil important in poultry.

Clinical trials in humans have not found any changes in platelet aggregation with flaxseed. Edel et al. (2016) also reported no change rate and degree of increased platelet aggregation by increasing the dose of flaxseed from 10 to 40 g/day in food. Another study also demonstrated flax seed prevents leukocyte and platelet adhesion to endothelial cells in rats (Haliga et al., 2013). In another clinical study, it was reported that a flaxseed diet for 12 months had no effect on heart function, heart rate and heart diastolic duration. (Caligiuri et al., 2014).

In this study, no differences were observed between the control group and groups that *flaxseed* was added to their *feed* in terms of blood and tissue values. Only the number of heartbeats was lower than the control group (Table 2) but this value of 310±23 is similar to values reported for poultry in literature (Emre et al., 1994; Çınar et al., 1996; Çınar et al., 2006) and it was among the reference values.

In this study, the averages of the mean electrical axis of the heart were found as -82 (-60-120), -90 (-55- -120), -85 (-58- -115) and -84 in groups (I, II III and IV), respectively. These values show parallelism those were reported by Çınar et al., (1996), Çınar and Dönmez (2001) and Çınar et al., (2006). It can be interpreted as the heart proceeds forward and right in the broilers studied. It has been shown that the incidence of subclinical heart diseases is high in fast-growing broilers and that the deaths occurring in relation to these diseases lead to significant economic losses (Baghbanzadeh and Decuypere, 2008). Blood parameters are related to the health status of the animals (Çınar et al., 2006), and have a diagnostic importance in assessing the general state of the animal.

CONCLUSION

In conclusion, even if the additional nettle seed, grape seed and flaxseed (30 mg/kg) to broiler rations did not have any positive effect on body weight gain, it is also important that it has no negative effect on blood, heart, liver, kidney, lung and spleen. This state indicates that these substances do not cause any pathological circumstances in the case of adding to broiler ration and these can be used physiologically. The results suggest that the presence of these substances at certain levels is necessary for blood production and normal metabolic activities in the animals and these have positive effects on carcass quality and substantiality.

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Role of glial fibrillary acidic protein (GFAP) and neurofilament (NF) expression in the pathophysiology of canine distemper encephalomyelitis

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ABSTRACT

Objectives: Canine distemper virus (CDV), a member of the genus *Morbillivirus* of the family *Paramyxoviridae*, is the causative agent of canine distemper, a fatal and highly contagious disease that affects dogs and other carnivores. This study aimed to investigate whether there is a correlation between glial fibrillary acidic protein (GFAP) and neurofilament (NF) expression in canine distemper encephalomyelitis (CDE) and the severe neuropathology that occurs.

Materials and Methods: GFAP and NF expression levels in the brain tissue of 13 dogs diagnosed with CDE were investigated by immunohistochemical method.

Results: The results of the study revealed that GFAP ($p< 0.005$) and NF ($p< 0.005$) expression levels in brain tissue were significantly increased in CDV-infected dogs compared to healthy, uninfected dogs. GFAP expression was mainly observed in endothelial cells and astrocytes, whereas NF expression was mainly found in neurons. In addition, it was found that the expression of both GFAP and NF was more pronounced in the areas with the most severe neuropathological findings.

Conclusions: This study demonstrated pathological astrocyte reactivation and neuronal degeneration at the molecular level. These findings provide information about the stage of the disease. This study clearly demonstrated that detailed information about the prognosis of the disease can be obtained from GFAP and NF expression. Since GFAP/NF levels provide information about the severity of the disease, they can be used clinically. Therefore, further research into the involvement of GFAP and NF expression in the pathophysiology of CDE has great potential to improve our understanding of this complex neurological disorder.

Keywords: *Canine distemper, GFAP, NF, Neuropathology, Encephalomyelitis*

INTRODUCTION

Canine distemper virus (CDV) is a single-stranded, negative-sense *Morbillivirus* from the *Paramyxoviridae* family, closely related to the human measles virus. It is the etiological agent of fatal distemper disease in dogs (Barrett, 1999; Murphy et al., 2012). CDV's host range includes not only dogs and other canines, but also large felines (such as leopards and tigers), raccoons, coatimundi, giant pandas, ferrets, and rhesus monkeys. Although these animals are not generally considered susceptible, they can be experimentally infected (Dalldorf et al., 1938; Qiu et al., 2011; Beineke et al., 2015; Martinez-Gutierrez and Ruiz-Saenz, 2016). In addition to neuropathology in both grey and white

matter in distemper, demyelinating leukoencephalomyelitis is considered the main neuropathological finding in dogs (Summers and Appel, 1987; Summers and Appel, 1994; Beineke et al., 2009).

Glial fibrillary acidic protein (GFAP) is considered the main intermediate filament protein in astrocytes and the building block of the cytoskeleton (De Zeeuw and Hoogland, 2015). In addition to maintaining the integrity and permeability of the blood-brain barrier, astrocytes regulate water transport via aquaporins and provide vital support to neurons/oligodendrocytes (Abbott et al., 2006; De Zeeuw and Hoogland, 2015). Astrocytes and microglia have been shown to be involved in neurodegeneration processes by rapidly and actively responding to damages occurring in neurons (Laping et al., 1994; Mongin and Kimelberg, 2005). In neural parenchymal pathology, astrocytes become activated and increase their number, size, and GFAP expression (Laping et al., 1994; Mongin and Kimelberg, 2005). Particularly, astrocytes play significant roles in CNS trauma, ischemia, parasitic (Dincel and Atmaca, 2015), viral (Dincel and Kul, 2015), and metabolic encephalopathy (Dincel and Yıldırım, 2016), and a significant upregulation in GFAP expression is a remarkable finding (Hol and Pekny, 2015). Additionally, increased GFAP expression is directly proportional to cellular hypertrophy, the degree of cellular reactivity, and especially neuropathology (Sofroniew, 2014).

When analyzing demyelinating distemper lesions, it is seen that the main target of CDV is astrocytes. In fact, it has been found that almost 95% of the infected cells are astrocytes (Summers and Appel, 1987; Mutinelli et al., 1989). Astrocyte hypertrophy, gemistocyte astrocytes (reactive astrocytes), and astrocytic syncytia formation observed in canine distemper encephalomyelitis (CDE) has been shown to be closely related to progressive myelin loss (Vandevelde et al., 1982; Summers et al., 1984; Mutinelli et al., 1989). The functional significance of astrocyte plasticity in canine distemper has not yet been fully clarified.

Neurofilaments (NF) are a basic component of the neuronal cytoskeleton and are in constant interaction with neighboring glial cells (Julien and Mushynski, 1998; Al-Chalabi and Miller, 2003). Pathological accumulation of NF is a key finding in many neurodegenerative diseases, including amyotrophic lateral sclerosis, Parkinson's, Alzheimer's, Charcot-Marie-Tooth, dementia with Lewy bodies, Border Disease, and Toxoplasmic Encephalitis (Al-Chalabi and Miller, 2003; Liu et al., 2004; Dincel and Atmaca, 2015; Dincel and Kul, 2015). Abnormal NF accumulation is mainly associated with axonal degeneration and plays a role in differentiating neuronal dysfunction (Al-Chalabi and Miller, 2003; Liu et al., 2004). Furthermore, NF release into the cerebrospinal fluid is an crucial indicator of the severity of neuropathology in neurodegenerative diseases (Norgren et al., 2003). These studies demonstrate that NF expression is closely related to neuropathology.

In this study, it was planned to show the severity of neuropathology seen in CDE by GFAP and NF expressions. Therefore, it is aimed to investigate the correlation between GFAP/NF expressions and their role in neuropathogenesis and to have information about the neurodegeneration levels of the disease with the findings obtained. It is thought that the findings obtained will give an idea for the treatment process of the disease.

MATERIALS and METHODS

Ethics Statement

This study was approved by Atatürk University Rectorate, Faculty of Veterinary Medicine, Unit Ethics Committee with the decision numbered 2023/25. The study samples comprised 13 dead dogs, ranging in age from 1 to 3 years. These animals were brought to Aksaray and Atatürk University, Faculty of Veterinary Medicine, Department of Pathology for routine necropsy. No animals studied were sacrificed for this study, and all procedures were performed with the permission of the animal owners. A total of 6 distemper-negative dogs without brain pathology and CNS-related cause of death were used as healthy control animals.

Pathologic examination

No macroscopic or histopathological findings were found in the brain tissues of 6 healthy control dogs. All steps followed the procedure described by Dincel (2017). The brains were removed and fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) at pH 7.4 for 48 h and then were thoroughly rinsed overnight, under tap water. After performing the routine tissue preparation procedures of dehydration using graded alcohol and xylene, the tissue samples were embedded in paraffin blocks; 4–5 μm thick paraffin

sections were then cut and mounted on glass slides. Hematoyxlin - Eosin (H&E) and immunohistochemical tests were performed, and they were analyzed using a trinocular light microscope (Olympus BX51 and DP25 digital camera). The severity of CDE in each animal was classified according to neuropathological changes: demyelination, hyperemia, mononuclear cell infiltrates, gliosis, astrocytosis, neuronal degeneration and malaise.

Antibodies

Commercial antibodies against GFAP (Abcam, Cambridge, UK) diluted to 1:250 and undiluted NF (Thermo Scientific, USA) were used.

Immunoperoxidase examinations

Immunohistochemistry was performed to observe GFAP and NF in the 4–5 μm-thick paraffin sections of the tissues by using an indirect streptavidin/biotin immunoperoxidase kit (HRP, Thermo Scientific, USA), as per the manufacturer's instructions. All steps were carried out following the procedure described by Dincel and Kul (2019). Briefly, the sections were placed onto adhesive slides, deparaffinized for 5 min. Each in the 3-step xylene series, and rehydrated using a series of graded alcohol and distilled water. The antigens were retrieved by boiling the tissue sections on glass slides in citrate buffer (pH 6.0) (Thermo Scientific, USA) for 20 min. Endogenous peroxidase activity was quenched using 3% hydrogen peroxide in absolute methanol for 7 min at room temperature (RT). The tissue sections were rinsed thrice with phosphate buffer solution (pH 7.4) for 5 min, between each consecutive step. The sections were then incubated in a blocking serum for 5 min to prevent non-specific antibody binding. Thereafter, the sections were incubated with GFAP and NF antibodies for 60 min in a humidity chamber at the RT. After treating the sections with biotin-labelled secondary antibody for 15 min and streptavidin-peroxidase enzyme for 15 min at RT, the colour reaction was performed using 3,3'-Diaminobenzidine chromogen for 5-10 min. Sections were counterstained with Mayer's hematoxylin for 1–2 min and suspended in a water-based mounting medium (Thermo Scientific, USA). For each immunoperoxidase test, three negative control tissue sections were allowed as follows; as a negative control, one of the serial paraffin sections was incubated with normal mouse serum (isotype serum control) instead of primary antibody. Additionally, the primary antibody step was omitted to control non-specific endogenous peroxidase and biotin activities in each test.

Histomorphometric analysis and statistics

The density of positive staining was measured using a computerized image system composed of a Leica CCD camera DFC420 (Leica Microsystems Imaging Solutions, Ltd., Cambridge, UK) connected to a Leica DM4000 B microscope (Leica Microsystems Imaging Solutions, Ltd.). Five representative fields were selected, and consecutive pictures were captured by Leica QWin Plus v3 software under a 20x objective lens (Leica Microsystems Imaging Solutions, N Plan) at a setting identical to the imaging system for analysing. We used the same setting for all slides. The integrated optical density of all GFAP and NF-positive staining were measured, and the mean GFAP and NF-positive area/total area was calculated by Leica QWin Plus v3. All images were collected under the same lighting conditions. To avoid observer bias, a blinded investigator quantified all sections. Data were described in terms of mean and standard deviation (mean ± SD) for area %. After calculating the proportion (% pixels) of the stained area to the whole field, each slide's mean (% pixels) staining area was determined. GFAP and NF immunohistochemical results were compared between groups using a mann whitney u test. $p<0.005$ was considered statistically significant.

RESULTS

Histopathologic findings

Although severe findings are seen in the cerebellum, lesions in brain tissues were analysed in this study. H&E-stained brain sections from healthy control animals exhibited normal architecture. Significant histopathological lesions were observed in the brain tissues of all animals. Histopathological examinations revealed areas of demyelination in the brains of the dogs, which were not very diffuse and appeared as moth-eaten areas (Figure 1A). Marginal hyperchromasia (Figure 1B) and intranuclear inclusion bodies were observed in some cells. Astrocytosis, astrogliosis and microglial cell proliferations were observed close to the areas of demyelination. Gitter cells were also found in areas with severe neurohistopathological findings. Diffuse hyperaemia and neuronal degeneration were detected (Figure 1AB). The observation of liquefaction necrosis in the parts where the lesions

were severe was considered as an important finding.

Distribution of viral antigens in the brain sections

It has been shown that all parts of the brain, including the cerebral hemisphere, cerebellum, neutrophil and midbrain, are strong diffuse and/or granular labelling of CDV antigens. In addition, linear or diffuse dense immunopositive staining was observed in endothelial (Figure 1C), neurons (Figure 1C) and glial cells (Figure 1D).

Immunoperoxidase findings

We analysed the protein expressions levels of GFAP and NF in the brain tissues from CDV-infected and healthy control animals. Immunohistochemical analysis showed significant up-regulation of GFAP and NF expressions in the CDV-infected dogs (Figure 2) unlike that in the case of the healthy control animals. Statistical analysis of the data on GFAP and NF expressions in the brain, measured by immunostaining in all the groups, are listed in Table 1.

Table 1. Immunoperoxidase test results and statistical data for healthy control and Distemper + animals

Animals	n	GFAP		*p*	NF		*p*
		Mean	SD		mean	SD	
Healthy Control Animals	6	0.726	0.367	0.001	1.727	0.312	0.001
Distemper + Animals	13	5.137	0.531		6.956	0.668	

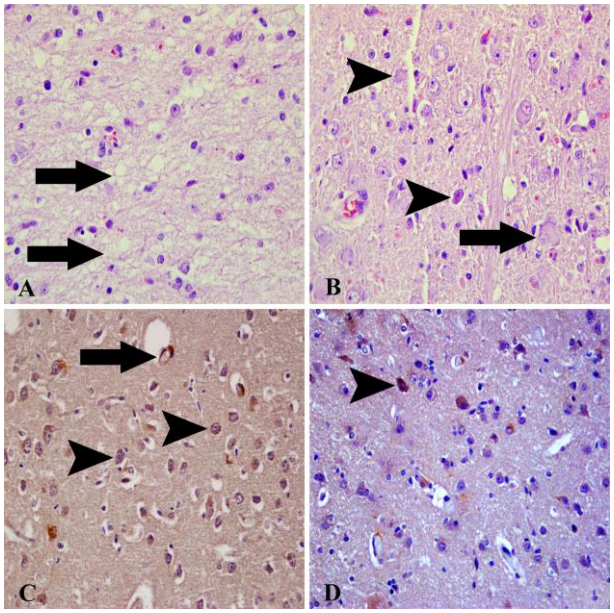

Figure 1. Large areas of moth-eaten demyelination (arrows). (A) Necrotic neurons (arrowheads) and marginal hyperchromasia (arrow). (B) Note the positive immunolabelling (red pigment) in the cytoplasm of degenerative/necrotic neurons (arrowheads) and endothelial cells (arrow). ABC technique (anti-CDV), Mayer's haematoxylin counterstain. (C) Note the positive immunolabelling (red pigment) in the cytoplasm of degenerative/necrotic glial cells (arrow). ABC technique (anti-CDV), Mayer's haematoxylin counterstain. (D)

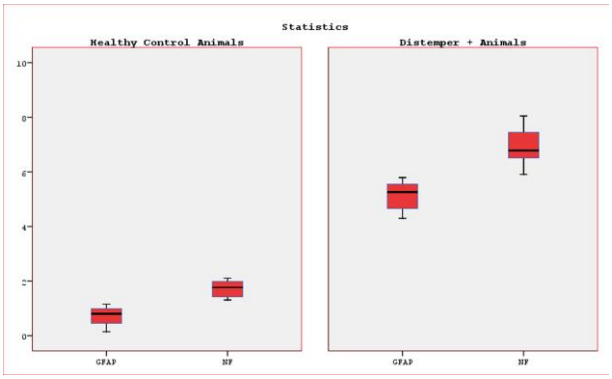

Figure 2. Significant up-regulation of GFAP and NF expressions in the CDV-infected dogs.

GFAP expressions

Fairly weak immunoreactivity for GFAP was observed in endothelial cells and neurons in healthy control animals (Figure 3A). GFAP expressions were observed especially in endothelial cells (Figure 3CD). An important finding is that these endothelial cells consist of degenerated cells. In addition to these findings, the appearance of GFAP-positive astrocytes close to necrotic neurons was considered an important finding (Figure 3BCD). The number of GFAP-expressing hypertrophic astrocytes increased in a virus-associated manner at the border of the focal gliosis. As a striking finding in the regions where viral antigens were concentrated, GFAP expressions were found to be strongly positive. There was a statistically

significantly higher incidence of positive GFAP immunoreactivity in astrocyte, neuron and endothelial cells than the levels in the healthy control animals ($p<0.005$).

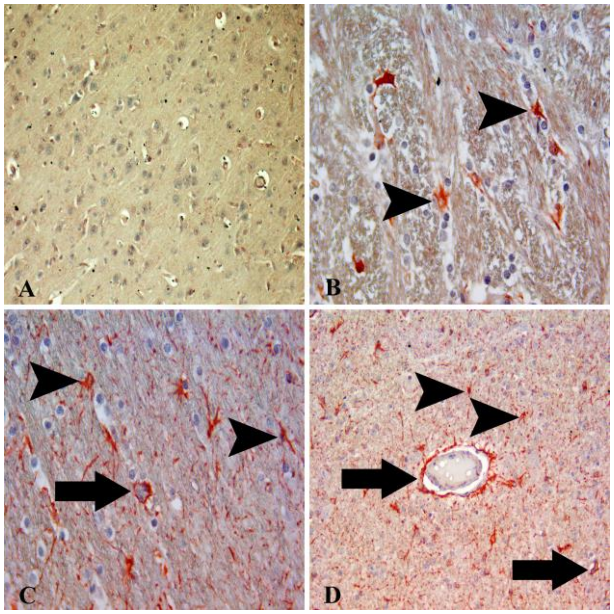

Figure 3. Weak GFAP expression in glial, endothelial cells and neurons of healthy control animals. ABC technique (anti-GFAP), Mayer's hematoxylin counterstain. (A) Strong GFAP expression in hypertrophic astrocytes (arrowheads). ABC technique (anti-GFAP), Mayer's haematoxylin counterstain. (B,C) Strong GFAP expression in hypertrophic astrocytes (arrowheads). ABC technique (anti-GFAP), Mayer's haematoxylin counterstain. (B,C) Strong GFAP expression in endothelial cells (arrows). ABC technique (anti-GFAP), Mayer's haematoxylin counterstain. (C,D)

NF expressions

Fairly weak immunoreactivity for NF was observed in the brain parenchyma and neurons in healthy control animals (Figure 4A). Abnormal NF accumulation and axonal degeneration were observed in the brain tissue of animals infected with CDV. NF was disorganised and significantly increased in some neurons and neuronal perikarya (Figure 4 BCD). The regions of increased NF immunoreactivity were localised within the lesion (demyelinated and focal gliosis area) and especially around it. Massive, regional accumulation of NF was noted in areas surrounding the cerebral blood vessels. Likewise, it is a remarkable finding that NF expressions show strong positivity in regions where viral antigens are concentrated. There was a statistically significantly higher incidence of positive NF immunoreactivity in the brain parenchyma and neurons than the levels in the healthy control animals ($p<0.005$). This pathological increase was significantly more pronounced in CDV-infected than in healthy control dogs. The increase in GFAP and NF expressions closer to the lesion areas is thought to be important evidence of neural parenchymal degeneration.

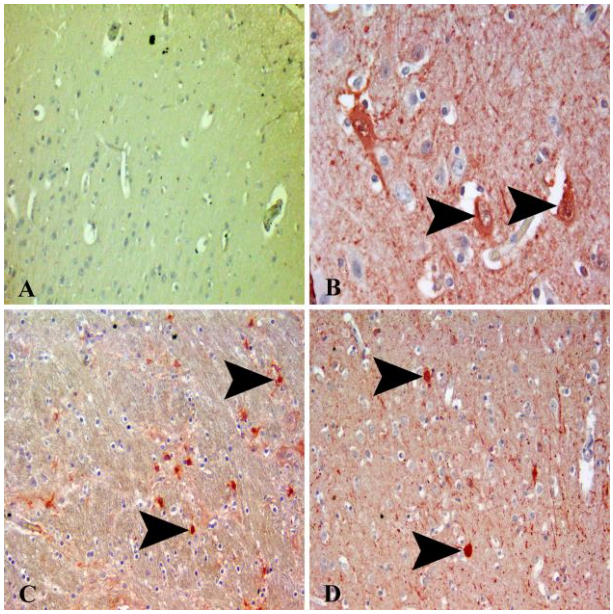

Figure 4. Weak NF expression in brain parenchyma and neurons of healthy control animals. ABC technique (anti-NF), Mayer's hematoxylin counterstain. (A) Strong NF expression in neurons (arrowheads) and brain parenchyma. ABC technique (anti-NF), Mayer's haematoxylin counterstain. (B,C,D)

DISCUSSION

CDE is a fatal disease with a poor prognosis due to severe neuropathology following infection with CDV, a *Morbillivirus* from the *Paramyxoviridae* family. Although there have been studies on the neuroimmunopathogenesis of distemper, molecular pathogenesis is still not fully understood. It remains a useful model for many demyelinating diseases. In this study, we investigated GFAP and NF expressions in CDE and revealed their correlation with observed neuropathology. The most striking finding of this study is that GFAP and NF expressions are positively correlated with neuropathology. Additionally, we found that GFAP and NF expressions are positively correlated with each other, which is another important discovery. These findings provide important insights into the neurodegeneration levels of the disease and could inform its treatment process.

Astrocytes play very important roles, including supporting neuronal function, providing communication between neurons, maintaining cerebral homeostasis, and regulating neuronal synapses (Perea et al., 2009; De Zeeuw and Hoogland, 2015). When any damage occurs in the neural parenchyma, GFAP expressions are upregulated, making it reactive against neurodegeneration (Laping et al., 1994; Mongin and Kimelberg, 2005). In this study, all CDE cases showed significant GFAP expressions, indicating the involvement of the increase in GFAP expression in the neurodegenerative process. This is a clear indication of moderate or high-level damage to astrocytes when evaluated together with histopathological findings.

NFs are a vital component of the neuronal cytoskeleton, and they interact with the surrounding glial cells to maintain healthy neuronal functions (Barrya et al., 2007; Yuan et al., 2012). Abnormal accumulation of NF occurs primarily in damaged neurons (Al-Chalabi and Miller, 2003; Norgren et al., 2003). In cerebral and neurodegenerative diseases with acute neural parenchymal destruction, pathological NF accumulation indicates the severity of neuropathology and can aid in diagnosis (Norgren et al., 2003; Liu et al., 2011). In this study, we detected a severe accumulation of NF at significant levels in CDE cases. Like GFAP expressions, the high levels of NF accumulation in cases with severe neuropathology are an important finding of the study. This finding clearly demonstrates the presence of severe neural parenchymal destruction and demyelination with neuronal degeneration at the molecular level. Therefore, it is evident that NF expressions will provide valuable information about the onset and progression of the disease.

CONCLUSION

In conclusion, this study demonstrated high levels of astrocyte reactivation and neuronal degeneration at the molecular level. The findings provide information about the stage of the disease, as GFAP/NF expressions were found to be directly proportional to the level of neural parenchymal destruction and neuropathology. Therefore, GFAP and NF expressions can be used to obtain detailed information about the prognosis of the disease. It is believed that these biomarkers can be used clinically since GFAP/NF levels can give information about the severity of the disease.

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The effect of evening primrose oil on some biochemical parameters in brain tissue in a model of metabolic syndrome induced with fructose in rats

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ABSTRACT

Objective: Metabolic syndrome is a disease characterized by hypertension, dyslipidemia and insulin resistance, and constitutes an important risk factor for cardiovascular disorders. The effect of evening primrose oil (EPO) on insulin, adiponectin and resistin levels in brain tissue was investigated in a fructose-related metabolic syndrome model.

Materials and Methods: The rats were divided into 4 groups as control, evening primrose oil (orally at a dose of 0.1 mLrat/day), fructose (20% fructose added), fructose+evening primrose oil for 57 days. At the end of the experiment, brain samples were taken and homogenized. Then, insulin, adiponectin and resistin in brain tissue levels were determined by ELISA.

Results: Plasma insulin and resistin levels of the fructose group increased ($p \leq 0.05$) compared to the controls, on the contrary, adiponectin levels were significantly decreased ($p \leq 0.05$) in the fructose group. When EPO was given to rats given fructose, increased insulin and resistin levels decreased (2.54 ± 0.28^{a}, 2.12 ± 0.68^{a}), (2.21 ± 0.26^{b}, 2.04 ± 0.21^{a}) while decreased adiponectin levels were increased (0.64 ± 0.42^{c}, 1.02 ± 0.35^{b}).

Conclusion: It was observed that the impaired metabolic changes caused by fructose in the brain tissue were partially improved in the EPO-treated group as a result of the decrease in insulin, resistin and increase in adiponectin. Accordingly, since metabolic changes in the brains of rats fed with high fructose content may also occur in humans with fructose intake from various foods, the use of EPO in the medical setting may be recommended by clinicians to reduce the harmful effects on the brain.

Keywords: *Brain, Fructose, Rats*

INTRODUCTION

Since human metabolism is regulated by glucose, fructose is not a sugar suitable for humans. In addition, fructose and its technological products contribute to obesity, diabetes and the formation of some cancer types, fructose causes lipogenesis, dyslipidemia and hepatosteatosis. It is reported to be caused by imbalance (Levine et al., 2003). Consumption of multiple fructose-sweetened beverages and foods has also been documented to cause hepatosteatosis, visceral obesity, and decreased sensitivity to insulin. As a result of increased consumption of foods, fructose intake has reached 60-150 gr daily. It is estimated that this amount meets 10.2% of the daily energy need. Soft drinks sweetened with high fructose corn syrup constitute the majority of dietary fructose (Malik et al., 2010).

Today, hypertension, dyslipidemia and insulin resistance occur as a result of the metabolic syndrome caused by many exogenous and endogenous factors, and cardiovascular diseases and type 2 diabetes develop. In addition to these findings, it is observed that fructose causes lipid peroxidation in various tissues and oxidant enzyme activities are increased by polymorphnuclear leukocytes infiltrating the tissues (Rayssiguier et al., 2006; Crescenzo et al., 2013; Bircan, 2014).

The oil obtained from the seed of primrose (*Oenothera biennis* L), a wild medicinal flower, contains different vegetable oils but is rich in gamma linolenic acid. It is used in traditional medicine in many different parts of the world to treat many internal and external diseases. Commercially, evening primrose oil and different vegetable oils containing γ-linolenic acid (GLA) are sold in encapsulated form as food supplements. Evening primrose oil is known to favorably modify a deteriorating lipid profile (Singer et al., 1986; Villalobos et al., 1998; Abo-Gresha et al., 2014).

The brain is a metabolically very active organ. Significant energy is used for events such as regulating ion concentration in synaptic transmission, generation of electrical potentials, active uptake of excitatory neurotransmitters and synthesis processes in the brain. The brain uses glucose, an important metabolic substrate, as its main energy source (Herculano-Houzel, 2011). Glucose metabolism in the brain is oxidative, with most of the glucose being oxidized to carbon dioxide and water. Complete oxidation of glucose to carbon dioxide and water may not always occur. This is called the glycogen pathway, and it is important for astrocytic glycogen stores (Reagan et al., 2002; Shah et al., 2012).

Adiponectin is thought to play an important role in the modulation of glucose and lipid metabolism in insulin-sensitive tissues in both humans and animals. Decreased circulating adiponectin levels have been demonstrated in genetic and diet-induced mouse obesity models of obesity (Yamauchi et al., 2001).

Resistin is known to be produced in adipose tissue in mice. It has been hypothesized that resistin level may be a triggering factor of diabetes and obesity-related metabolic disorders in mice (Urbanovych et al., 2015). Resistin is an in vitro insulin antagonist in human preadipocytes. Overexpression of resistin, which has been shown to be proinflammatory cytokines in recent studies, by liver cells impairs glucose uptake and glycogenesis. Positive correlations with proinflammatory factors have been demonstrated in adults with pathophysiological conditions such as atherosclerosis, kidney disease, respiratory tract inflammation, and type 2 diabetes mellitus (DM2) (Jinhua, 2012).

In this study, the effect of evening primrose oil on insulin, adiponectin and resistin levels in brain tissue was investigated in a fructose-induced metabolic syndrome model.

MATERIALS and METHODS

The animal material of this study was obtained from Van Yüzüncü Yıl University Experimental Animals Unit. Forty male Wistar albino rats, 12-16 weeks old, weighing 250-300 g, were used. During the experiment, the rats were housed in rooms with 12 hours of dark/lighting and a temperature of 22±2°C, in cages with constant feed and fresh water in front of them. Standard rat food, drinking water or tap water with 20% fructose was given ad libitum. The study was carried out in accordance with the code of ethics, and with the approval of the local ethics committee of the animal experiments of Van Yuzuncu Yil University (Decision number: 31.08.2023, 2023/10-06).

Experimental applications

Animals were randomly divided into 4 groups. Trial period is 57 days. Control group (10 rats): Standard rat food and tap water were given.

Evening primrose oil group (EPO) (10 rats): Standard rat feed and tap water were given. In addition, evening primrose oil (Biotama, Turkey) was administered orally by gavage at a dose of 0.1 mL/rat/day (Kaya, 2010) for 57 days.

Fructose group (10 rats): Standard rat food and tap water with 20% fructose added (Bircan, 2014) were given for 57 days.

Fructose+Evening Primrose Oil Group (Fructose+EPO) (10 rats): Evening primrose oil was administered orally at a dose of 0.1 mL/rat/day, with standard rat food and tap water with 20% fructose added, for 57 days.

Taking the samples

After the experimental application, only water was given and the animals were fasted overnight. The rats were given 90 mg/kg of ketamine i.p., after the animals were sacrificed, the brain was immediately removed and weighed. It was kept in a deep freezer at -80 degrees.

Frozen brain tissue samples were weighed after thawing and homogenized in cold 0.1 M phosphate buffer (pH=7.4) solution in a homogenizer (Heidolph Slient Crusher M, Bear, Delaware United States) (He Supernants were obtained by centrifugation at 4500 rpm for 20 minutes at 4°C (Mohamed et al., 2015).

In the resulting supernatant, Rat Insulin ELISA Kit YL Biont (Item No.: YLA0037RA), Rat adiponectin, ELISA Kit YL Biont (Item No.: YLA0076RA), Rat Resistin ELISA Kit YL Biont (Item No. YLA0203RA), ELISA kits, a Examined using a Statfax 2600 automatic washer and a Statfax 2100 reader.

Statistical analysis

"SPSS Statistic 20" package program was used in the analysis of the data. Kruskal Wallis test was used for statistical analysis of all parameters. Results with less than $p<0.05$ are considered to be statistically significant between groups.

RESULTS

Effects of evening primrose oil on some parameters in brain tissue in rat with fructose-induced metabolic syndrome were shown in Table 1.

Insulin levels of brain tissue in the fructose group were found to be higher than those in the control and EPO groups. This surplus decreased when EPO was added to the fructose given group. However, there was no statistically significant difference between the insulin level changes of both the control and fructose groups. However, it is seen that EPO reduces the insulin-raising effect of fructose.

Adiponectin level in the brain tissue was found to be significantly lower in the fructose group than in the control and EPO groups. This decline increased when EPO was added to the fructose given group. A statistical significance was found between the changes in fructose and adiponectin levels of the other groups.

The level of resistin in the brain tissue followed a similar path to other parameters. Again, it was found to be significantly higher in the fructose group than in the control and EPO groups. This increase decreased when EPO was added to the fructose group. Statistically significant changes were found between the resisitin level changes of fructose and other groups (Table 1).

Table 1. Brain tissue levels of insulin, adiponectin and resistin in metabolic syndrome in rats induced with fructose.

Parameters	Control X±SD	EPO X±SD	Fructose X±SD	Fructose+EPO X±SD	*P*
Insulin (µg/g tissue)	1.98±0.23^{a}	1.64±0.91^{a}	2.54± 0.28^{a}	2.12±0.68^{a}	0.098524 NS
Adiponectin (µg/g tissue)	1.29±0.98^{a}	1.133±0.52^{a}	0.64±0.42^{c}	1.02±0.35^{b}	0.000129 *
Resistin (pg/g tissue)	1.91±0.22^{a}	1.65±0.35^{c}	2.21±0.26^{b}	2.04±0.21^{a}	0.002526*

a,b,c: The differences between values containing different letters in the same line were found to be statistically significant at the $p\leq0.05$ level. Evening primrose oil (EPO).

DISCUSSION

In laboratory animals fed a high fructose diet, some disorders paralleling metabolic syndrome markers have been identified. Similarly, in humans, it has been observed that first of all, hypertension and insulin resistance are formed, followed by impaired glucose metabolism and uptake pathways, followed by an increase in triglyceride synthesis and lipogenesis (Bircan, 2014; Arslan and Şanlıer, 2016). For this reason, giving high fructose diets to experimental animals is critical for establishing metabolic syndrome and observing changes, identifying disease-causing mechanisms and developing new strategies for disease prevention/treatment (de Moura et al., 2008).

Bircan (2014) added 20% fructose to the drinking water of rats for 8 weeks. At the end of this period, when the fructose administered group was compared with the control group, it was determined that it caused a statistically significant increase in systolic blood pressure, serum insulin and triglyceride levels, insulin resistance developed and metabolic rate was increased. Created the syndrome model in rats (Bircan, 2014).

Liver in metabolic syndrome induced by fructose diet; It is the organ that is most affected by the metabolic changes and is the earliest damaged due to both being the main organ responsible for fructose metabolism and its function in carbohydrate and lipid metabolisms (Grattagliano et al., 2008; Bagul et al., 2012).

The extracellular glucose concentration in the blood is important in the transport of glucose to the brain tissue. Short-term hyperglycemia, which occurs when the amount of glucose in the blood plasma increases compared to normal values as a result of different foods and disorders in the organism, or long-term hyperglycemia, as in diabetes (Horani and Mooradian, 2003). Some of these metabolic pathways constitute the indirect pathway of passive responses independent of neuronal activity. In addition, it has been shown that the active responses of hyperglycemia in gene expression in neurons in the central nervous system have a very important place in neuronal damage (Klein and Waxman, 2003).

It has been observed that magnocellular neurosecretory cells in the hypothalamic supraoptic and paraventricular nuclei increase vasopressin release against the increased hyperosmolarity as a result of chronic glucose increases (Dheen et al., 1994). These cells contain mechanosensitive voltage-gated sodium channels that detect changes in extracellular osmolarity. The continuation of the hyperosmolar state causes "up-regulation" of sodium channels and a decrease in the threshold value for the generation of the action potential with the increase in the number of sodium channels. Encephalopathy, which manifests itself with cognitive and behavioral disorders, may occur as one of the late complications of diabetes. The incidence of progressive dementia due to chronic hyperglycemia in people with diabetes is also high (Grober et al.,2011). Increased free radical production, vasogenic edema, decrease in cerebral blood flow as a result of short-term or long-term hyperglycemia, as well as changes in gene expression in central nervous system cells cause neuronal and endothelial damage. In fact, hyperglycemic states that reach behavioral disorders in animals were also observed when fructose was given, and in the brain tissue analyzes performed, insulin, adiponectin and resistin levels were found to be high in hiperglycemic group (de Moura et al., 2008). According to our results, metabolic syndrome occurred in rat given fructose for about 2 months significant biochemical changes were observed in high fructose group.

During fructose metabolism, pyruvate production is faster because the phosphofructokinase step, which is one of the rate-limiting enzymes in glucose metabolism, is skipped. The pyruvate formed is converted into triglycerides in the liver rather than energy production by the Kreps cycle. Therefore, increased fatty acid synthesis can increase circulating fatty acids and stored fat. This may lead to lipotoxicity, which reduces the insulin sensitivity of cells due to the production of fatty acids in tissues other than adipose tissue (Neilson, 2007). In this study, while insulin levels in the brain tissue were 1.98 μg/g tissue in the control group, it was measured as 2.54 μg/g tissue in fructose rats. In the group given fructose+EPO, this value decreased to 2.12 μg/g tissue, showing the positive effect of EPO.

The presence of increased levels of glucose in the brain due to high fructose nutrition or different reasons has been reported in humans and different experimental animals (Dorn et al., 1983). Insulin can cross the ENT, CSF levels of mice increased slightly after peripheral infusions of this hormone, suggesting that insulin probably crosses the ENT via a saturable transport system. The system of transport of insulin from the blood to the brain is affected by certain factors such as glucocorticoids or in various pathophysiological conditions such as hunger and obesity, during aging as well as hibernation. It can be regulated in individuals with diabetes mellitus (Blázquez et al., 2014). As a result of post-mortem biochemical brain analyzes, the presence of C-peptide and immunoreactive insulin was detected in cadaver brain tissue (Young, 1986). These substances were detected in the highest amount in the hypothalamus, while their brain levels were much higher than in the blood. Based on these findings, it can be argued that the insulin detected here is a component of the brain itself. Because, as a result of studies at the molecular level, the presence of insulin mRNA was found in the CA1 and CA3 regions of the hippocampus, the dentate gyrus, and the granule cell layer of the olfactory organ (Devaskar et al., 1994). Hyperglycemia and subsequently increased insulin level were detected in the brain tissue. It is worth repeating that, with the effect of lowering blood glucose and insulin levels (Mert et al., 2022), EPO has a positive effect on glycemia and a lowering effect on brain insulin level was observed (Table 1). The reversal of the increase in blood glucose, that is, proving the hypoglycemic effect of EPO, by administering evening primrose oil to streptozotocin-induced diabetic rats in previous years, was also shown in this study when high fructose was administered and EPO supplementation was made, the results are consistent with those of the studies known in the literature (Söğütlü et al., 2019). In the presented study the levels of insulin were increased in

fructose group, addition of EPO slightly decreased the insulin amount but statistical importance were not observed between the research groups.

Adiponectin, an endocrine substance synthesized and released from adipose tissue (Yamauchi et al., 2001), has an important role in the regulation of carbohydrate and fat metabolism in insulin-sensitive tissues. While it reduces glycogenesis in the liver, it increases the oxidation of fatty acids and glucose uptake in muscle tissue (Fruebis et al.,2001). Adiponectin is anti-diabetic, antiatherogenic and anti-inflammatory. Since they have a role in (Al-Rashed et al., 2016; Kamari et al., 2007) deficiency, some metabolic disorders and subsequent diseases develop. In addition, these theoretically mentioned issues have been proven clinically in different studies (Arita et al., 1999) Experimentally, biochemical analyzes performed on the metabolic syndrome created at the end of high fructose nutrition yielded similar results to our study. In the aforementioned studies, it was reported that adiponectin level decreased (Kamari et al., 2007; Shokouh et al., 2017), insulin level and insulin resistance increased (Tran et al., 2009; Zhou et al., 2013). Hyperinsulinemia was shown to decrease circulating adiponectin levels (Yu et al., 2002). In this study, adiponectin levels were found to be significantly decreased in rats fed high fructose compared to controls. It is observed that the level of adiponectin increases significantly with the application of EPO, and the level of insulin decreases with the application of EPO (Table 1). Our findings is consistent with the findings Zhou et al. (2013).

Adiponectin is a hormonal regulator that facilitates the transition of free fatty acids from circulation to fat cells. In cases where the storage capacity of the adipose tissue decreases, such as obesity, and/or, in cases where the existing energy resources, such as increased energy requirement in the periphery, such as acute inflammation, are desired to be transferred to the peripheral tissue instead of being stored in the adipose tissue, the serum level is down-regulated and decreases (Fu and Luo, 2005)

Coronary artery disease (CAD) is an important complication frequently encountered in diabetic patients. While plasma adiponectin levels were found to be lower in individuals with CAD than in diabetic patients without CAD, these results show that adiponectin may have antiatherogenic properties. In addition, low adiponectin levels have been associated with atherogenic lipid profile in clinical studies. It is reported that adiponectin has a role in increasing the tyrosine phosphorylation of the insulin receptor, which is known to contribute to the increase of insulin sensitivity. Thus, a series of biochemical processes follow each other that will contribute positively to the regulation of the lipid profile in the liver. These are metabolic phenomena such as decreased free fatty acid mobilization and increased fatty acid oxidation, decreased hepatic glucose output and very low density lipoproten (VLDL) triglyceride synthesis (Chandran and Phillips, 2003)

Adiponectin is a plasma protein of 30 kDa with a molecular weight of 247 amino acids synthesized from adipose tissue. This cytokine is a collagen-like protein within the soluble collagen superfamily (Scherer et al., 1995; Kishore and Reid, 2000) As previously explained, adiponectin has prominent anti-inflammatory and antiatherogenic effects, especially in macrophages and endothelial cells. Again, it can be said by looking at the data that this substance plays a protective role in the pathological events that occur at the beginning of the disorder in the formation of atherosclerosis and vascular damage models, and the disorders are shaped in decreasing amounts.

Fu and Luo (2005) reported that low plasma adiponectin levels are associated with the development of obesity, insulin resistance, and cardiovascular disease. They suggested the existence of a peroxisome proliferator–activated receptor γ (PPAR-γ)-mediated mechanism on the observed changes. Other researchers have reported that some seed oils in the diet have the ability to activate PPAR-ɣ, resulting in adiposity, decrease in leptin, and increase in adiponectin (McFarlin, 2009).

Abo-Gresha et al. (2014) administered evening primrose oil to rats suffering from hypercholesterolemic myocardial infarction for 6 weeks in order to examine the antithrombotic, anti-inflammatory and cholesterol-lowering effects of EPO. Based on the findings, they found that EPO had a direct hypocholesterolemic effect and caused cardiac recovery through its indirect effect on the synthesis of eicosanoids.

Metabolic syndrome, which occurs experimentally or with diseases in animals and humans, is closely related to oxidative stress. Nikotinamid adenin dinükleotit fosfat hidrojen (NADPH) oxidase and possibly adipocyte mitochondria are the main source of reactive oxygene species (ROS) in experimental metabolic syndrome, and it has been proven that oxidative stress stimulates insulin

resistance in adipocytes. It has been determined that the decrease in adiponectin production in adipocytes is caused by oxidative stress, and obesity, which is shaped by malnutrition and some hormonal disorders, has also been shown to trigger oxidative stress. Oxidative stress is not only a consequence of the metabolic syndrome, its role is an important factor and essential link in the pathogenesis of the metabolic syndrome (Tran et al., 2009; Maslov et al., 2018). As a result of the detection of oxidative stress in fructose fed rats, feeding with a high fructose diet was associated with increased oxidative stress and the development of insulin resistance (Bloch-Damti and Bashan, 2005; Delbosc et al., 2005), when these rats were treated with antioxidants, ROS production was found to decrease and insulin resistance was inhibited (Song et al., 2005). Increased reactive oxygen species and increased uric acid levels have been reported to contribute to fructose-induced hypertension (Tran et al., 2009).

A new hormone called resistin (insulin resistance), which has been linked to obesity and type 2 diabetes, was first found in mice and later in humans (Berger, 2001). Following resistin's first identification in 2001, several important discoveries were reported (Stephan et al., 2001). Among these, plasma resistin levels can be stimulated by diet. Presence of induced and genetic forms in obese mouse models; increased insulin sensitivity by administration of anti-resistin antibody in obese or insulin-resistant animals; New findings include treating healthy mice with impaired glucose tolerance and insulin action with recombinant resistin and that resistin administration impairs insulin-induced glucose uptake in adipocytes. From these observations, it was concluded that resistin plays an important role in insulin resistance and obesity in the diabetic mouse model. Determining the usability of the findings in human studies has also been difficult. While resistin is secreted from white adipose tissue in mice, it has been reported that it is synthesized from circulating blood monocytes and to a lesser extent from white adipose tissue in humans (Savage et al., 2001). In many studies conducted in humans to date, it has been reported that there is a positive correlation between elevated serum resistin level and obesity, insulin resistance and obesity. First, resistin has been reported to be expressed in human hepatocytes and induce insulin resistance (Sheng et al., 2008). Furthermore, human resistin mRNA levels were found to be higher in peripheral blood mononuclear cells compared with female patients with type 2 diabetes (DM2) and healthy women, suggesting a role for resistin in the pathogenesis of human DM2. In some rodent models, resistin mRNA expression in adipose tissue of obese animals has been shown to be uncorrelated with serum resistin levels that do not correlate with serum insulin or glucose.It has been reported that both increases and does not change (Lee et al., 2003). In this study, resistin levels elevated in the fructose group decreased slightly when EPO was given to rats in the fructose group. Statistical significance was observed between the fructose group compared to the others.

CONCLUSION

Metabolic syndrome is characterized by hypertension, dyslipidemia and insulin resistance, an important risk factor for cardiovascular disorders and type 2 diabetes, has been established experimentally. The examined parameters were selected from substances that undergo significant changes in metabolic disorders. It has been suggested that the administration of fructose begins to increase in a way that leads to the formation of insulin resistance by increasing the level of insulin, that the decrease in the level of adiponectin is an important parameter in the development of obesity and insulin resistance, and that the resistin level may also be a triggering factor for diabetes and obesity-related metabolic disorders. In support of this information, insulin and resistin levels were found to increase in brain tissue in this study, and levels decreased with EPO administration. On the contrary, the level of adiponectin decreased in fructose-treated rats, while the administration of EPO increased the level of adiponectin in brain tissue. Fructose-induced impaired metabolic changes in brain tissue were partially ameliorated when EPO was administered. Accordingly, the use of EPO in the medical setting may be recommended by clinicians to reduce the harmful effects on the brain, since metabolic changes in the brains of mice fed with high fructose content can also occur with the intake of fructose from various foods in humans.

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Mucins: an overview of functions and biological activity

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ABSTRACT

This review aims to provide novel evidence on the function of mucins in defense of epithelia and to spot mucin changes in the epithelial surface.

High molecular weight glycoproteins known as mucins are distinguished by their substantial O-glycosylation. The membrane-bound mucins and in secretion form are divided into two categories mucins. These are among the significant mucins expressed by the surface epithelia. Recent developments in functional assays have evaluated their functions in preserving corneal, conjunctival, respiratory, and digestive epithelia. The presentation includes changes in mucin and mucin O-glycan production in epithelial surface illnesses, including infection, non-autoimmune dry eye, autoimmune dry eye, and allergy.

Mucins are high molecular weight glycoproteins characterized by their extensive O-glycosylation. Recent advances using functional assays have allowed the examination of their roles in protecting epithelial tissues. Alterations in mucin and mucin O-glycan biosynthesis in epithelial surface disorders, including allergy, non-autoimmune dry eye, cancers, and infection, are presented.

Keywords: *Classification of mucins, Functions of mucins, Mucins*

1. Introduction

Mucins (MUC) are a large macromolecular component of mucus composed of glycoproteins. It is dispersed along the epithelial surface of the digestive, respiratory and reproductive systems (Rachagani et al., 2009). The mucin synthesis begins in the granular endoplasmic reticulum and is completed in the Golgi apparatus. Mucins are stained weakly with acidophilic dyes such as hematoxylin-eosin (Bancroft and Gamble 2008). Mucins are structurally and functionally divided into two. These are sekrete mucins and membrane mucins. Mucins that form the secretion are MUC2, MUC5AC, MUC5B, MUC7, and MUC6. Membrane mucins are MUC1, MUC3, MUC4, MUC12, MUC13, MUC16, MUC17, and MUC20 (Fowler et al., 2001; Pang et al., 2022).

MUC1 plays a role in preventing the adhesion of proteins; MUC3 has a role in defending the body against pathogens (Brayman et al., 2004), MUC4 and MUC13 protect epithelial cells (Pelaseyed et al., 2014; Corfield, 2017), MUC12 enables signal transduction (Yakan, 1990), MUC16 facilitates pancreatic cancer progression and metastasis (Aksoy, 2001), MUC17 allows cells to cling to each other on the apical surface of the tissue (Önder, 2012), MUC20 maintains the humidity of epithelial cells on mucosal cell surfaces (Woodward and Argüeso., 2014), MUC5AC supports the balance of bodily fluids in organs such as the colon and stomach (Bonser and Erle, 2017), MUC2 is involved in the diagnosis of ovarian tumors (O'Connell et al., 2002), MUC7 plays a role in the secretion of mucins at a specific limit in the glycosylation mechanism

(Büyük, 2014), MUC5B serves in the acquisition of viscoelastic properties of mucins (Ratan et al., 2021), MUC6 plays a role in protecting organs in the gastrointestinal tract (Fowler et al., 2001; Matte et al., 2019). Finally, MUC19 plays a role in ensuring the humidity of the respiratory tract (Das et al., 2010).

2. Mucins (MUC)

Mucins are among the first glycoproteins identified as biological compounds in the body. It has been reported that mucins are found in the saliva of nesting birds, such as swallows, and play an important role in the function of saliva and that sugars join the structural parts of mucin glycoproteins (Karaçalı, 2003; Corfield, 2017; Dhanisha et al. 2018).

In humans, it is found in the apical cell membranes of tissues such as the digestive, respiratory tract, and urogenital systems. In general, mucus is secreted to protect the respiratory, gastrointestinal, and reproductive tracts (Fowler et al., 2001; Bryd and Bresalier, 2004; Matte et al., 2019).

2.1. Structure of Mucins

Mucins are glycoproteins synthesized by epithelial cells. Its structure contains oxygen-dependent serine/threonine/proline-rich proteins. They are structures that bind with peptides lined up one after the other and have a high oligosaccharide content and high molecular weight. Mucins in a dense glycosylated state are among the macromolecules with viscoelastic properties. Mucins, which make up the main structure of mucus, are compounds that have all the properties of a protein and the properties of sugars under certain conditions (Aksoy, 2001; Joshi et al., 2018; Argüeso, 2022).

Mucins secreted by epithelial cells mature as they move from the base of the crypt of the luminal organs to the lumen. This maturation manifests itself in the form of a decrease in lysosomes, an expansion of the Golgi membranes, and an increase in the endoplasmic reticulum and mucin-secreting vesicles. The peptic core part of the mucin is collected in the ribosomes of the granular endoplasmic reticulum. From here, it is transported by non-granular endoplasmic reticulum channels and comes to the vesicles in the Golgi apparatus. After this stage, glycosylation occurs in the Golgi apparatus (Corfield, 2017; Svensson, 2018).

2.2. Functions of Mucins

The main function of mucins is to ensure protection and the lubricity of the luminal organs in the body. Mucins contribute to the adhesion of epithelial cells, differentiation, and renewal of cells by providing signal modulation. Membrane-bound mucins function as cell surface receptors for pathogens and help activate intracellular signaling pathways (Bonser and Erle, 2017, Bansil and Turner, 2018).

Mucins are involved in all physiological events as heavily glycosylated proteins, including inflammation, activation of the immune system, and tumor formation (Birchenough et al., 2015; Haugstad et al., 2015; Tassew et al., 2022).

In the glycosylation mechanism, mucins facilitate cell adhesion during tumor metastasis. It regulates the immune system's response, and it has also been reported that mucin can displace proteins according to the functions of proteins in the interaction of carbohydrates (Chaturvedi et al., 2008; Corfield, 2017).

2.3. Classification of Mucins and Areas of Action of Mucins

It has been stated that mucins are divided into membrane-bound mucins and secreted mucins. Secreted mucins are divided into gel-forming and non-gel-forming mucins have also been stated. (Fowler et al. 2001; Cha et al. 2015; Oh et al., 2015).

Table 1. Classification of mucins (Fowler et al. 2001; Cha et al. 2015; Oh et al. 2015).

Mucins		
Secretory Mucins		Membrane Associated
Gel-forming	Non-gel-forming	
MUC 2	MUC 7	MUC1
MUC 5AC	MUC 8	MUC3
MUC 5B	MUC9	MUC4
MUC 6		MUC10
MUC 19		MUC12
MUC 11		MUC13
		MUC16
		MUC17
		MUC18
		MUC20

2.3.1. Membrane-associated mucins

Membrane mucins are mucins in glycoprotein structure containing peptide domains, and it has been stated that they protect epithelial cells by being on the apical membrane of epithelial cells, provide cell interactions and play a role in signal transmission between cells with their cytoplasmic extensions (Van and Strijbis 2017).

Table 2. Histochemical staining for determination of mucins (Alan and Liman 2010).

Paints	Identified Mucin	Color
PAS	Neutral mucins, weak sulfate mucins	Purplish, red
PAS/D	Neutral mucins	Red
AB (ph2,5)	Sulfomucin ve sialomucins	Blue
PAS/AB, PAS	Neutral mucins	Red
(ph 2,5), PAS/AB	Mixture of neutral and acidic mucins	Purple
HID	Sulfomucin	Black/ Dark Brown
HID/AB, AB (ph 2.5)	sialomucins	Blue
(pH 2.5), HID/AB	Mixture Sulfomucin and sialomucins	Bluish brown
AF	Sulfomucin	Purple
AF/AB, AB (ph 2.5)	sialomucins	Blue
(pH 2.5), AF/AB	Mixture Sulfomucin and sialomucins	bluish purple
PAPS	Neutral mucins	No Staining
PAPS	Sialomucins	Purplish Red

PAS: Periodic Acid Schiff, **PAS-D**: Periodic Acid Schiff-Diastase, **AB-PAS**: Alcian Blue-Periodic Acid Schiff, **HID-AB**: High İron Diamine-Alcian Blue, **AF-AB**: Aldehyde Fuchsin-Alcian Blue, **PAPS**: Periodic acid- Phenylhydrazine-Schiff

2.3.1.1. MUC1

It is one of the subunits of the protective family of mucins and one of the mucins that are rich in serine and contain threonine deposits with intense O-glycosylation properties around proteins. They prevent proteins from sticking together. MUC1 is secreted from the apical part of the epithelial cells of many organs. In cancer cells, it occurs not only in the apical but also in the lateral or cytoplasm of the cell membrane (Cheever et al., 2009; Lakshminarayanan et al., 2016; Yousefi et al., 2019).

MUC1 is involved in the protection of cells by providing hydration of cell surfaces. MUC1 is an effective inhibitor in intracellular and extracellular matrix interactions. MUC1 has a highly dynamic structure in normal epithelial cells, which changes in response to the effects of steroid hormones or cytokines. The MUC1 gene is found as heavily glycosylated mucin on the apical surfaces of the simplest epithelial cells, including the urogenital system, GIS (Gastrointestinal system), respiratory system, and some non-epithelial cell types (Sakurai et al., 2007; Wu et al., 2018; Khodabakhsh et al., 2021). Mucins in the lungs have been secreted from goblet cells and mucous and serous cells in the submucosal glands (Kufe, 2009; Bafna et al., 2010; Menon et al., 2015).

2.3.1.2. MUC3

MUC3, one of the membrane-bound transmembrane mucins, is on the apical side of epithelial cells. They are macromolecules that protect epithelial tissues against harmful microorganisms with endogenous and exogenous origin by enveloping the cells in contact with the external environment, like capsules (Ho et al., 2006; Kumar and et al., 2022). MUC3 is present in excess in goblet cells and enterocytes of the small intestine (Lakshminarayana et al., 2016; Ratan et al., 2021).

It has been reported that MUC3 increases in the epithelial part of the appendix in cases of appendix cancer and plays a role as a determining factor in patients with appendix cancer (Shibahara et al., 2014).

2.3.1.3. MUC4

MUC4, also known as the Sialomucin complex or SMC, is more weakly expressed in tissues such as the respiratory tract, colon, cornea, female genital tract, and breast. It is rich in serine and threonine (Dharmaraj et al., 2014; Bansil and Turner, 2018). MUC4 begins to be synthesized in the digestive tract at 6.5 weeks of pregnancy, and its release increases with the increase of progesterone. It is found only in the trachea between 8-12 weeks of pregnancy. However, after the 12th week of pregnancy, it gradually increases in the small bronchi and bronchioles. It has been observed that it exists in the epithelial cell of the jejunum on the colon axis and the epithelial cells of the esophagus. It has been reported that there is an association between MUC4 and the differentiation to the squamous cell carcinoma stage. It has also been observed that MUC4 is not present in the liver, bile ducts, gallbladder, and pancreas during pregnancy. In adults, it is released in large quantities in

secretions of body parts such as the respiratory system, endolymph, and breast milk (Koscinski et al., 2006; Chaturvedi et al., 2008).

2.3.1.4. MUC11

MUC11 is a type of mucin produced from tandem repeats of 28 amino acids formed by the combination of serine, threonine, and proline. MUC11, which is one of the membrane-bound mucins, has been detected in the middle ear, thymus, lung, colon, pancreas, prostate, and uterus (Williams et al., 2001). The epithelial surface has various functions, including protection from pathogens that may cause infection, communication through intracellular signaling, cell differentiation, and cell proliferation (Hijikata et al., 2011). It is also thought to play a role in cystic fibrosis disease in lung tissue. It has been shown to prevent the adhesion of epithelial cells to each other in malignant tumors (Fowler et al., 2001; Hernandez-Jimenez et al., 2008).

2.3.1.5. MUC12

It is a type of mucin rich in proline, which is in the form of successive repetition of degenerate amino acids and has 28 amino acids in its structure. It is a methionine consisting of serine protein that contains epidermal growth factor and similar domains rich in extracellular cysteine. MUC12 is responsible for epithelial cell protection, adhesion modulation, and signal transduction (Önder, 2012; Alcântara et al., 2022).

2.3.1.6. MUC13

It is a membrane glycoprotein with high molecular weight. It has been reported that it is at a moderate level in the large intestine, trachea, and kidney, with the highest level in the small intestine. Also, it is crossover in stomach and mouse tissues and is found at an intermediate level in intestinal epithelial and lymphoid cells in situ hybridization (Pelaseyed et al., 2014; Pang et al., 2022).

MUC13 protein has been expressed in the GIS system and in the trachea's apical membrane of prismatic and goblet cells. The MUC13 protein is broken down into monomers in the GIS system and divided into two subgroups. The cytoplasmic tail of the protein containing the ß subunit, one of the monomer subgroups of the MUC13 protein, regulates gene expression in the nucleus by stimulating the protein kinase C signaling pathway (Williams et al., 2001; Corfield, 2017; Kumar et al., 2022).

2.3.1.7. MUC15

MUC15, a member of the mucin family, is a glycosylated substance found in lymphoid organs (thymus, spleen, bone marrow, etc.), placenta, testis, ovary, small intestine, colon, etc. has been identified as a transmembrane protein (Zhang et al.,2020).

2.3.1.8. MUC16

It is membrane-bound mucin and is also called CA125, which is a heavily O-glycosylated transmembrane protein (Giamougiannis et al., 2021). MUC16 is used to detect cystic fibrosis and various types of cancer. MUC16 has been found on the epithelial surface of the cornea, conjunctiva, respiratory tract, reproductive tract of the female, and on the epithelium of the tracheal surface as a component of the ocular surface (Aithal et al., 2018; Argüeso, 2022).

MUC16, together with MUC1 and MUC4, is involved in intercellular communication and intercellular signaling. MUC4 takes advantage of MUC16 to ensure that the cell surface glycocalyx of the ocular layer in the eye acquires hydrophilic properties. It protects the eye from foreign particles and infectious diseases. In addition, it helps to open and close the eyelid by ensuring the wetness of this area. In this way, it prevents dry eye disease (Menon et al., 2015; Lakshminarayanan et al., 2016; Li et al., 2018; Martens et al., 2018; Matte et al., 2019).

2.3.1.9. MUC17

In addition to gel-forming mucin, it is found on microvilli in the GIS system (small and large intestine), which are among the transmembrane mucins and have a brushy appearance. Microvilli here ensure the capture of foreign substances. It has been reported that it helps to protect against harmful toxins in the GIS system and to provide intracellular vesicle localization in epithelial cells. The carboxy-extremity of MUC17 in the GIS system contains a hydrophobic domain. This feature allows the cells to stick to each other on the apical surface of the tissue (Sakurai et al., 2007; Önder, 2012).

2.3.1.10. MUC18

MUC18 is also known as CD146. MUC18 is a transmembrane glycoprotein with a length of 113-kDa. MUC18, first found in malignant cells in humans, has also been detected in smooth muscle and endothelial cells in the airway wall of lung tissue in subsequent studies. It is noted that it defends the body against foreign microorganisms by activating T lymphocytes in the lung. It has been

determined that it protects the body against pathogens by creating an inflammatory response to pathogens in patients with COPD (Chronic obstructive pulmonary disease) and asthma (Simon et al., 2011; Sun et al., 2016).

2.3.1.11. MUC20

It has been revealed that it is responsible for the protection of epithelial cells on mucosal surfaces. It has a unique localization in the cell layers between multilayered epithelium cell types and throughout the epithelial cells of the human cornea and conjunctiva. During MUC20 differentiation, it is thought to be secreted from the ocular surface and to play an important role in keeping the wetness of this place in balance and maintaining this balance (Bafna et al., 2010). In epithelium ovarian cancer (EOC) cells, it activates integrin ß1 and provides signal transduction. In this way, it provides a new perspective on the role of signaling by resisting pathogens (Woodward and Argüeso. 2014; Chen et al., 2016).

2.3.2. Secretory Mucins

It has been reported that they can be divided into gel-forming and non-gel-forming mucins (Cha et al. 2015).

2.3.2.1. Gel-Forming Mucins

It has been stated that it has an viscoelastic feature, which has a supporting role in mucosal defense (Oh et al. 2015).

2.3.2.1.1. MUC2

It contains serine, proline, and threonine in its structure. It is intestinal-type secretory mucin mainly synthesized in goblet cells. It is considered to be biochemical insoluble mucin that causes high viscosity in the region where it is synthesized (Büyük, 2014; Birchenough et al. 2015 Liu et al., 2020).

It has been reported that MUC2 release in the lung is observed especially in mucinous adenocarcinomas (colon cancer) and its release increases in tumors related to the GIS system (Wang and El Bahrawy, 2015; Astashchanka et al., 2019; Liu et al., 2020). It is a marker that can be used in the diagnosis of ovarian tumors. It is secreted in mucinous tumors of the appendix and abdominal region. It has also been noted that it is the most secreted mucin in breast cancer (O'Connell et al., 2002; Lau, et al., 2004; Astashchanka et al., 2019).

2.3.2.1.2. MUC5AC

MUC5AC forms an internal mucus layer in organs such as the colon, pancreas, and stomach for the regulation of body fluids. It has been determined that it plays a role in protecting organs by increasing hydrochloric acid. It is gel-secreted mucin released from goblet cells in the lungs, eyes, and stomach, and was first reported to be detected in the middle ear (Büyük, 2014; Val et al., 2015; Bonser at al., 2017; Ratan et al., 2021).

MUC5AC has been reported to increase in endocervical cancers and has also been observed in other adenocarcinomas, endometrium, and lung adenomas (Lau et al., 2004; Bonser and Erle et al., 2017; Okuda et al., 2019).

2.3.2.1.3. MUC5B

It is one of the macromolecular proteins containing polymers, monomers, and glycopeptides in its structure. It has been reported that this mucin is secreted in saliva, normal lung mucus, and the cervical region, making a significant contribution to the viscoelastic property of the structures here (Val et al., 2015; Joshi et al., 2018; Ratan et al., 2021).

MUC5B mucin has been detected in regulated chronic rhinosinusitis (CRS), chronic obstructive pulmonary disease (COPD), a gastric disease associated with *Helicobacter Pylori,* and sinus mucosa diseases and has been reported to play a role in the pathogenesis of these diseases (Wang and El-Bahrawy, 2015; Evans and et al., 2016; Hughes et al., 2019).

2.3.2.1.4. MUC6

It occurs in Brunner's glands and pancreatic ducts during 18-19 weeks of pregnancy. Its presence was determined also in the gastric glands during the 20th week of pregnancy. It has been reported to be present in the stomach mucosa, the gallbladder, seminal vesicles, pancreatic centroacinar cells, and the periductal area of the bile duct. It may have the function of preserving epithelial tissues (Wang and El-Bahrawy, 2015).

MUC6-related diseases: pancreatic ductal carcinomas and bile papillomatosis (Matte et al., 2019).

2.3.2.1.5. MUC19

It is among the most recently discovered gel-forming mucins. It is present in the submucosal glands and within the secretion in the middle ear (Kerschner et al., 2009; Kumar et al., 2022).

It is a type of mucin that acts as an exocrine in the sublingual mucus and salivary glands in mice. MUC19 plays a role in maintaining the wetness of the respiratory tract and against irritations caused by nutrients (Das et al., 2010).

2.3.2.2. Non-Gel-Forming Mucins

It has been stated that it is divided into two as MUC7 and MUC8 (Dhanisha et al., 2018).

2.3.2.2.1. MUC7

The increase of MUC7 in the glycosylation mechanism leads to mucins settling in an abnormal order, increasing the potential for tumor invasion and metastasis (Büyük, 2014). It is secreted from the mucous cells of the submandibular and sublingual salivary glands and is thought to give the saliva a viscous property (Önder, 2012). The presence of MUC7 has been detected in respiratory secretions in asthmatic patients and pediatric patients. Thus, it prevents foreign bodies from entering the body in the respiratory system (Ratan et al., 2021).

2.3.2.2.2 MUC8

It was first detected in 1994 thanks to a successive series of repeating cDNA tandems (Shankar and et al., 1994). It contains many cysteines and consecutive serine and threonine in its structure. It has been reported that it helps to secrete mucus for healing respiratory diseases by stimulating an adipocytokine synthesized by white adipose tissue in the epithelial cells of the respiratory tract. It has also been found that MUC8 increases in the lungs of cystic fibrosis patients (Finkbeiner et al., 2011; Cha et al., 2015).

2.3.2.7. MUC9

MUC9, also known as Oviductin, is secreted in large quantities in the female's oviducts by the influence of the estrogen hormone. It is responsible for the protection of the oviducts of the female and the developing embryo. It is also considered to play an important role in the development of the embryo. It has also been reported to be a marker for the diagnosis of ovarian cancer (Hendrix et al., 2001; Laheri et al., 2017). MUC9 is thought to have positive effects on sperm capacity, motility, and viable cell in sperm in mammals. It has also effects on sperm-egg fusion and ovum penetration (Zhao et al., 2022).

2.4. Histochemical Classification of Mucins

They are divided into two, including acidic mucins and neutral mucins (Yakan, 1990; Ali et al., 2012).

A. Acidic mucins

Sulfate Mucins (Sulfomucin)

- Strongly sulfated acidic
- Strongly sulfated epithelial mucin
- Atypical sulfated mucins

Carboxylated mucins (sialomucins)

- Carboxylated mucins
- Sulfated sialomucins
- Hyaluronic acid

B. Neutral mucins

2.4.1. Acidic Mucins

Acidic mucins are classified into two parts among themselves. It has been stated that acidic mucins play a protective role by preventing the passage of microorganisms, such as bacteria-originating viruses and fungi. In addition, it has been reported that they help leukocytes to be transported to the target organ in growth factors, cell development, and signal transmission (Saruhan et al., 2016).

2.4.1.1. Sulfate Mucins (Sulfomucin)

- **Strongly sulfated acidic** mucins are found in connective and supporting tissue and stain negatively with Periodic acid-Schiff (PAS) and positively with Alcian blue (Ph 2.5).
- **Strongly sulfated epithelial mucin** appears in the serous bronchial glands and is positively stained with PAS.
- **Atypical sulfated mucins** are located in the bronchial glands in the trachea and stained with Alcian blue (Ph 2.5) (Yakan, 1990; Anđelković et. al., 2009).

2.4.1.2. Carboxylated (Sialomucin)

- **Carboxylated mucins** are present in the salivary gland and small intestine and are negatively stained with PAS.
- **Sulfated sialomucins** are found in prostate cancer.
- **Hyaluronic acid** is present in goblet cells in connective tissue (Yakan, 1990; Anđelković et. al., 2009).

2.4.2. Neutral Mucins

It has been stated that neutral mucins, which are composed of various hexosamines combined with free hexose groups, contain mannose, galactose, and monosaccharides in their structure. It is of the epithelial type and is most commonly found in the Brunner glands, in the mucus secreted by the epithelial cells lining the stomach, and it is stained with alkaline dyes. It was also reported that they

did not react with alcian blue but gave a positive reaction with PAS (Suvarna et al., 2013; Ghiurce et al., 2021)

2.5. The Relationship of Mucins with diseases

2.5.1. The Relationship of Mucins with Cancer

The carbohydrate sequencing of mucins in the tissues of a healthy individual is happening linearly. But the carbohydrate sequencing of mucins in tissues that encounter cancer manifests itself in the form of intermittent disconnections. Because of these properties, it causes cancer cells to spread directly out of the tissue where the mucins are located or to other areas through blood and lymph vessels (Haugstad et al., 2015).

Mucoepidermoid carcinoma (MEC) is the most common tumor in the salivary gland. Shemirani et al. studied salivary gland tumors and analyzed MUC12, MUC13, MUC17, MUC18, and MUC19 genes in twenty-three patients using PCR and RT-PCR techniques. It was reported that MUC19 was 26% in normal tissue. Still, when encountered with tumor cells, the rate of MUC19 in the tissue increased by 65%, the distribution of MUC18 in tumor and normal tissues was equal, MUC12 and MUC17 mucin were not seen at all in MEC, MUC13 is at the rate of 0% in normal tissue without disease and increased by 13% when the disease was encountered, MUC1 and MUC4, on the other hand, increased 21 folds more in the case of illness compared to the normal tissues (Shankar et al., 1994, Shemirani et al.,2011).

2.5.2. The Relationship of Mucins Pancreatic Cancer

Pancreatic cancer is one of the most important diseases that ranks fourth in mortality in the world. It has been reported that the uncontrolled increase of MUC1MUC4, MUC5AC and MUC16, which are transmembrane and secretory mucins, in pancreatic tissue causes pancreatic cancer (Kaur et al., 2013).

2.5.3. The Relationship of Mucins Covid-19

Covid-19 disease was reported in 2019 as a highly contagious respiratory disease. It has been stated that mucins play an important role in the diagnosis of covid-19 disease. It was stated that especially the MUC5AC, MUC5B and MUC1 mucins were found to be excessively increased in sputum content in the trachea region of Covid -19 patients (Bose et al., 2020; Lu et al., 2021).

2.5.4. The Relationship of Mucins Asthma

It has been stated that asthma is one of the lung diseases characterized by the obstruction in the airways caused by the combination of many factors, including mucus-producing cells, lipids, and proteins (Mirershadi et al. 2020). It has been stated that MUC5AC and MUC5B in the respiratory tract play an important role. It has been reported that MUC5AC increase and MUC5B decrease cause acute asthma by creating airway obstructions (Welsh et al., 2017).

2.5.5. The Relationship of Mucins Ulcerative Colitis

It is an Inflammatory Bowel Disease characterized by persistent inflammation of the large intestine. It has been reported that MUC2 mucin is expressed in the colon in healthy individuals and individuals with ulcerative colitis. It has been stated that goblet cells in the large intestine play a role in MUC2 expression and excretion. It has been stated that goblet cells are decreased as a result of mucosal damage in ulcerative colitis disease, resulting in a decrease in mucin production. It has been suggested that microbes infiltrate the mucosa excessively, increase inflammation and cause disruption of the integrity of the mucosal barrier. It is stated that it may play a role in the occurrence of ulcerative colitis as a result of the lack of mucin production (Van et al., 2019; Bankole et al., 2021).

2.5.6. The Relationship of Mucins Dry Eye Diseases

Surface epithelial cells have been reported to secrete glycosylated membrane-associated mucins, such as the glycocalyx, MUC1, MUC4, and MUC16, which form a hydrophilic barrier for eye protection, lubrication, and homeostasis. It has been stated that galectin-3 interacts from the anterior surfaces of the conjunctiva and cornea thanks to these mucins glycosylation, preventing the entry of pathogens into the eye, reducing friction during clipping, and keeping the eye wet. It has been stated that it is associated with the emergence of dry eye disease as a result of defects in the production of mucins (Baudouin et al., 2019; Jin et al., 2022).

2.5.7. The Relationship of Mucins Dental Caries Diseases

It has been reported that MUC5B and MUC7 play a role in salivary mucins. It has been indicated that mucins bind to bacteria in the mouth and play a role in removing the bacteria from the mouth. For this reason, it has been pointed out that the decrease in

mucins in the mouth causes infections and inflammations, which plays a role in the thinning of the intraoral epithelial barriers, bleeding of the gums, and the emergence of dental caries (Linden et.al. 2009; Rusthen et. al., 2019).

CONCLUSION

Mucins must be investigated in more detail regarding providing moisturization of epithelial cell surfaces, epithelial cell renewal, and differentiation, intracellular signal transduction, cell adhesion, protection of body tissues against infections and injuries, as well as usability as a marker in the detection of cancer cells in some cancerous tissues.

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