Diameter and sarcomere length of skeletal muscle fibers in the tissue of the tongue during the developmental process of the sheep

Research Article

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ABSTRACT

The tongue plays an important role in the holding, chewing, and swallowing of food. This study was designed to determine the diameter and sarcomere length of skeletal muscle fibers in different parts of the tongue during the developmental process of sheep. For this purpose, small pieces of tissue were taken from the apex, the body, and the root parts of the tongue of sheep aged 6-12 months (G1, n:6), 1-2 years (G2, n:6), and 3-5 years (G3, n:6). Tissue samples were fixed in %10 formaldehyde solution and Crossmon's triple staining was applied to the tissue sections after routine histological processing. The diameter and sarcomere length of the skeletal muscle fibers in the tongue's apex, body, and root parts were measured. The diameter and sarcomere length of skeletal muscle fibers did not vary statistically between tongue regions in sheep in any age group ($p>0.05$). The sarcomere length of the skeletal muscle fibers in the apex, body, and root parts of the tongue did not change statistically with the development of the sheep ($p>0.05$). However, the diameter of skeletal muscle fibers at the apex and body parts of the tongue was statistically higher in G3 compared to G1 and G2, and the diameter of skeletal muscle fibers at the root part of the tongue was statistically higher in G3 compared to G1 ($p<0.05$). As a result, sarcomere length did not change throughout the development of the sheep, but skeletal muscle fiber diameter increased. Furthermore, skeletal muscle fiber diameter and sarcomere length did not vary between regions of the tongue.

Keywords: Fiber diameter, sarcomere length, sheep, skeletal muscle fiber, tongue

INTRODUCTION

The digestive system of ruminants involves a special process known as rumination, and the digestive process begins in the mouth. The tongue is located in the oral cavity and is responsible for collecting, chewing, and swallowing of food. Nutrients taken into the mouth are swallowed and sent to the rumen after a simple digestive process. With the help of muscle movement, water, and saliva in the rumen, the food is thoroughly mixed and broken down. Bacteria and other micro-organisms also digest and ferment food in the rumen. The tongue helps to mix the food in the rumen and makes fermentation more efficient. The food is then returned to the mouth, where it is chewed again, thoroughly soaked, and broken down into smaller pieces. Therefore, in ruminants, the tongue is very important for a healthy digestive process as it assists in food collection, preparation, and rumen fermentation and facilitates the swallowing process (Hofmann, 1989; Reddy & Hyder, 2023).

The tongue is a flexible organ. Its main structure consists of skeletal muscle fibers. The skeletal muscle fibers extend longitudinally, vertically,

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and transversely, giving the tongue the ability to move in multiple directions. The tongue is covered with stratified squamous keratinized epithelium. The lamina propria under the epithelium covers a narrow area. The submucosa contains serous, mucous, and sero-mucous lingual glands. The histological structure of the tongue is designed to support the functions of the tongue. For example, the mucosal layer provides protection against harmful substances from the external environment, while the tongue papillae and taste buds enable the ability to taste. The tongue muscles control the movement of the tongue and support functions such as speaking and swallowing (Gülmez, 2011; Tanyolaç, 1999).

The skeletal muscle fibers show transverse striations. Myofibrils are formed by the regular assembly of actin and myosin myofilaments, the two basic proteins of the skeletal muscle fibers. The light and dark areas formed by actin and myosin myofilaments are located along the myofibrils, and a transversely striped appearance is created by lining up areas of the same tone in the same line. The light-colored parts are called Isotropic (I) bands and the dark-colored parts are called Anisotropic (A) bands. A very thin and dark "Z" line is in the middle of the I band. The distance from one "Z" line to another "Z" line is known as the sarcomere (Ertbjerg & Puolanne, 2017; Kahraman et al., 2018). Sarcomeres are the functional unit of force generation in the skeletal muscle fibers, and sarcomere length is known to be an indicator of active muscle force (Son et al., 2018).

Understanding skeletal muscle growth and development is one of the most important goals in animal science. Therefore, muscle mass is mainly determined by the number and size of muscle fibers. However, the number of muscle fibers does not change after birth. During postnatal growth, the increase in skeletal muscle mass is mainly due to an increase in muscle fiber size (Rehfeldt et al., 2000). So, the measurement of muscle fiber diameter is often used as a parameter for skeletal muscle growth (Hegarty & Hooper, 1971).

Studies on the tongue in ruminants have focused especially on the tongue papillae (Can et al., 2016; Delibaş et al., 2023; Jabbar, 2014; Kurtul & Atalgın, 2008; Madkour & Mohammed, 2021; Mahdy et al., 2021; Tadjalli & Pazhoomand, 2004; Unsal et al., 2003). However, there are no studies on the development of the skeletal muscle fibers, which form the main structure of the tongue. This study aimed to reveal the diameter and sarcomere length of skeletal muscle fibers in the tongue depending on the development of sheep.

MATERIALS AND METHODS

Animal material and tissue processing

The tissue material used in this study was obtained from sheep brought to abattoirs in Siirt province for slaughter. Three different study groups were formed by collecting tongue tissues from sheep aged 6-12 months (G1, n:6), 1-2 years (G2, n:6), and 3-5 years (G3, n:6). Small pieces were taken from the apex, body, and root parts of each sheep's tongue. Tissue samples were fixed in % 10 formaldehyde (pH = 6.9-7.1) for 24 hours at room temperature. After routine histological processing, the tissues were blocked in paraffin. They were cut to a thickness of 5 microns. Crossmon’s triple staining was applied to tissue sections for histological examination and histometric measurements. Prepared sections were examined under a light microscope (DM750, Leica) equipped with a digital camera (MC170, Leica).

Histomorphometric measurements

Ten randomly selected areas from the apex, body, and root parts of each animal's tongue were photographed at 40x magnification. The diameter of 20 longitudinal skeletal muscle fibers was then measured separately from the apex, body, and root parts of each sheep’s tongue. In addition, the sarcomere length of the skeletal muscle fibers was measured, as described below. Sections containing at least ten sarcomeres from longitudinal skeletal muscle fibers were measured. Measurements were made from the beginning of the A-band to the end of the A-band of the other sarcomere. By counting the I-bands in the intermediate region, the number of sarcomeres was determined. The average sarcomere length was calculated by substituting the length of the measured region and the I band numbers into the formula below (Kahraman et al., 2018) (Figure 1).

Figure 1: Representative histometric measurements. blue line: Measurement of the diameter of the skeletal muscle fibers (13.75 µm, 10.70 µm), black line: The distance measured from the beginning of the A-band of one sarcomere to the end of the A-band of the other sarcomere (26.56 µm, 45.08 µm), the number of I-bands: (10 and 16). Crossmon's triple staining. Bar: 50 µm.

Average sarcomere length (µm) = Length of measured area (µm) / Number of I-bands.

In this study, a total of 1080 muscle fiber diameters and 1080 sarcomere length measurements were taken using Image J software (Image J, US National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

Statistical analysis of data was performed using Minitab® (v21.4.1). The Anderson-Darling normality test was used to assess the normal distribution of the data. Parametric tests were preferred because the data were normally distributed. One-way analysis of variance (ANOVA) was used to compare muscle fiber diameter and sarcomere length between age groups and parts of the tongue. The statistical significance level was evaluated as $p<0.05$.

RESULTS

Histological findings

The tongue of the sheep was covered with stratified squamous keratinized epithelium (Figure 2A). Skeletal muscle fibers, nerve plexuses, blood vessels, and lymph vessels were seen in the lamina propria and submucosa under the layer of epithelium (Figure 2A, B). Lingual glands and excretory ducts were observed in the body and root parts of the tongue, excluding the apex (Figure 2E-F). At the apex, body, and root parts of the tongue, the skeletal muscle fibers extended in the longitudinal, transverse, and vertical directions and constituted the majority of the submucosa (Figure 2B, C). The skeletal muscle fibers had numerous nuclei located just beneath the sarcolemma (Figure 2D). Transverse striations were noted in the skeletal muscle fibers, which extend in longitudinal and vertical directions (Figure 2D).

Histomorphometric findings

There was no statistically significant difference in the diameter of the skeletal muscle fibers between the apex, body, and root parts of the tongue in the G1, G2, and G3 groups ($p>0.05$) (Table 1). However, significant changes were observed in the diameter of the skeletal muscle fibers in the apex, body, and root parts of the tongue, depending on the development of the sheep. The diameter of the skeletal muscle fibers in the apex and body parts of the tongue was statistically higher in G3 compared to G1 and G2 ($p<0.05$) (Table 1). The diameter of the skeletal muscle fibers in the root part of the tongue was statistically higher in G3 compared to G1 ($p<0.05$) (Table 1).

Table 1: Diameter of skeletal muscle fibers in the apex, body, and root of the tongue of sheep aged 6-12 months (G1), 1-2 years (G2), and 3-5 years (G3).

Diameter of skeletal muscle fibers				
Groups	**Apex**	**Body**	**Root**	**p-value**
G1	14.86±0.61[c]	16.53±0.61[c]	16.60±0.53[c]	>0.05
G2	15.28±0.32[c]	17.36±0.42[c]	16.94±0.54[b,c]	>0.05
G3	17.21±0.56[b]	19.04±0.76[b]	18.43±0.20[a,b]	>0.05
p-value	0.013	0.035	0.017	-

[a,b,c]: different letters on the same column show significant differences between the mean values of the groups.

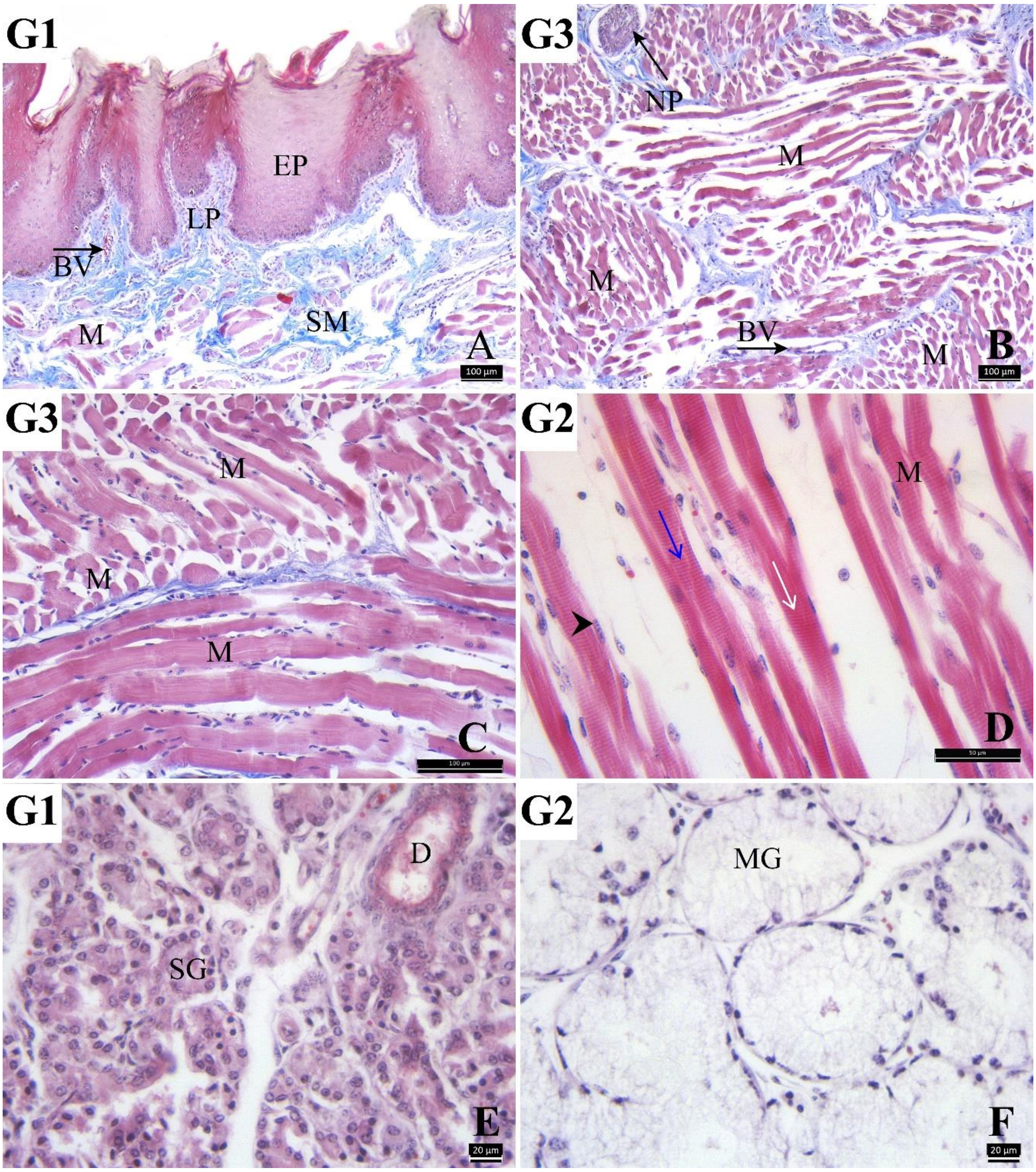

Figure 2: Histological appearance of the tongue of sheep aged 6-12 months (G1), 1-2 years (G2), and 3-5 years (G3). BV: blood vessel; D: excretory duct; EP: epithelial layer; LP: lamina propria; M: muscle fiber; MG: mucous gland; NP: nerve plexus; SM: submucosa; SG: serous gland; blue arrow: A band; white arrow: I band; arrowhead: nucleus. Crossmon's triple staining. Bar: A, B, C: 100 µm; D: 50 µm; E, F: 20 µm.

There was no statistically significant difference in the sarcomere length of skeletal muscle fibers between the apex, body, and root parts of the tongue in the G1, G2, and G3 groups (p>0.05) (Table 2). The sarcomere length of the skeletal muscle fibers in the apex, body, and root parts of the tongue did not change statistically with the development of the sheep (p>0.05) (Table 2).

Table 2: Sarcomere length of skeletal muscle fibers in the apex, body, and root of the tongue of sheep aged 6-12 months (G1), 1-2 years (G2), and 3-5 years (G3).

Sarcomere length of skeletal muscle fibers				
Groups	**Apex**	**Body**	**Root**	**p-value**
G1	2.66±0.02	2.58±0.06	2.78±0.06	>0.05
G2	2.69±0.05	2.60±0.08	2.76±0.03	>0.05
G3	2.57±0.03	2.68±0.05	2.70±0.05	>0.05
p-value	>0.05	>0.05	>0.05	-

DISCUSSION

The tongue, together with other organs in the oral cavity, plays an important role in the nutritional process (Steele & Van Lieshout, 2009). The tongue varies in size and shape in all mammalian species. Structural differences in the tongue between species reflect differences in the habitat and food resources of each species (Erdoğan & Sağsöz, 2018; Kadhim, 2016).

Erdoğan & Sağsöz, (2008) reported that the sheep tongue is surrounded by a multilayered keratinized epithelium and that blood vessels, nerve plexuses, and longitudinal, vertical, and transverse striated muscle fibers are present in the lamina propria and submucosa beneath the epithelial layer. Researchers detected lingual glands and excretory ducts in the body and root parts of the tongue except for the apex. In the present study, similar histological findings were observed in sheep tongues in all study groups.

Differentiated muscle cells lose their ability for division and proliferation (Sağlam et al., 2008). Previous studies have reported that there is no increase in the number of muscle fibers during postnatal development in mice (Rowe & Goldspink, 1969), rats (Brown, 1987; Rosenblatt & Woods, 1992; Schadereit et al., 1995), cattle (Wegner et al., 2000), chickens (Smith, 1963), and quail (Fowler et al., 1980). After birth, the total number of muscle fibers in mammals and birds does not change, and the increase in muscle mass occurs with the transverse and longitudinal growth of the myofibrils in the muscle fibers (Ertbjerg & Puolanne, 2017, Rehfeldt et al., 2000). Most muscle fiber growth takes place from birth to maturity (Ertbjerg & Puolanne, 2017). The ribosome organelle in muscle fibers synthesizes muscle proteins. These proteins are added to existing myofibrils and, when the myofibrils reach a certain thickness, they are split in the longitudinal direction. Thus, the number of fibrils in the muscle fibers increases and, accordingly, the diameter of the muscle fibers increases (Sağlam et al., 2008).

The sheep's tongue consists of the apex, body, and root parts (Kadhim, 2016). Al-Bazii et al., (2020) measured the height, width, and thickness of the apex, body, and root parts of the tongue of lambs and adult sheep. The researchers found that the height, width, and thickness of the tongue were statistically greater in adult sheep than in lambs. They stated that the sheep's tongue grows as its body grows.

There is no current study that shows the change in diameter of the skeletal muscle fibers, which form the main structure of the tongue, as the tongue grows during ruminant development. However, Fattah & El-Din, (2021) found that the diameter of the skeletal muscle fibers in the tongue tissue of old mice was significantly reduced compared to adult mice. In this study, the researchers examined the changes in the diameter of the skeletal muscle fibers in the tongue due to aging. With aging, degeneration of muscle fibers occurs due to changes in muscle fiber structure, decreased contraction ability of muscle fibers, and increased muscle fatigue (Kletzien et al., 2018). However, the present study revealed changes in the diameter of the skeletal muscle fibers in different parts of the tongue depending on the development of the sheep. In this study, it was determined that the diameter of the skeletal muscle fibers in the apex and body parts of the tongue was statistically increased in G3 compared to G1 and G2, and the diameter of the skeletal muscle fibers in the root part of the tongue was statistically increased in G3 compared to G1. These results indicate that the diameter of the skeletal muscle fibers in the tongue tissue increases as the sheep grow. However, while changes in the diameter of the skeletal muscle fibers due to the development of sheep showed similar characteristics in the apex and body parts of the tongue, the root part of the tongue showed different characteristics compared to the apex and body parts. It is thought that this situation is related to the development of sheep depending on their nutritional characteristics.

In a study conducted on the tongue of rats, it was found that the diameter of the skeletal muscle fibers increased as they moved away from the tip of the tongue toward the back (Cullins & Connor, 2017). The researchers detected that the skeletal muscle fibers in the anterior part of the tongue contract faster, while the posterior part of the tongue has a higher proportion of slower fibers. They have stated that smaller diameter muscle fibers in the anterior region of the tongue allow for motor control, while larger diameter muscle fibers in the middle and posterior regions of the tongue may contribute to bolus driving forces for propulsion into the hypopharynx (Cullins & Connor, 2017). However, in this study, it was found that the diameter of skeletal muscle fibers did not show a significant difference between the regions of the tongue throughout the development of the sheep. It is thought that this may be due to differences in dietary characteristics between species.

Sarcomeres are the smallest contractile units and serve as the primary force-generating machines of striated muscles. In skeletal muscle fibers, contraction is mediated by the interaction of actin and myosin myofilaments of sarcomeres arranged in series along contractile myofibrils. With the contraction, the skeletal muscle fibers are shortened and muscle strength is produced. Together with this basic mechanism, the skeletal muscle fibers perform various functions in the body (Gokhin et al., 2014).

The sarcomere length is a fundamental measurement that determines the ability of a muscle fiber to shorten and lengthen when it is contracted (Gokhin et al., 2014). The sarcomere length varies in a skeletal muscle-specific manner. Therefore, there are differences in sarcomere length between muscle types and animal species. In addition, there is no passive tension along a muscle fiber, so sarcomere length varies even within the same myofibril (Ertbjerg & Puolanne, 2017). In this study, the sarcomere length of the skeletal muscle fibers in sheep at different stages of development did not show statistically significant differences between regions of the tongue. According to this finding, it can be said that the skeletal muscle fibers in the sheep tongue have the same contractile capacity in different parts of the tongue to perform their functions.

Sarcomeres are constantly renewed as muscle fibers grow (Ertbjerg & Puolanne, 2017). However, it is known that the sarcomere lengths along a muscle fiber reach an optimum degree of filament overlap immediately after birth and remain unchanged thereafter (Williams & Goldspink, 1971). In a study conducted on the skin of mice, it was determined that the sarcomere length in different muscle types did not change with aging (Hooper, 1981). Similarly, in this study, it was determined that the sarcomere length of the skeletal muscle fibers in the apex, body, and root parts of the tongue did not change statistically during the developmental process of sheep.

CONCLUSION

It was found that the diameter and sarcomere length of the skeletal muscle fibers, which form the main structure of the tongue, did not differ between regions of the tongue in sheep of different ages. However, it was observed that as the sheep grew, the diameter of the skeletal muscle fibers in the tongue increased, but the sarcomere length did not change.

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Author contributions: BK designed the study, collected tissue samples, performed laboratory procedures, analyzed study data, and prepared the original draft of the manuscript.

Availability of data and materials: Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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