Praca oryginalna *Original paper*

Histomorphological characteristics of some muscles of Lika's Pramenka breed lambs in comparison with crossbreeds of Istrian Pramenka breed lambs

VESNA GJURČEVIĆ KANTURA, MLADEN ZOBUNDŽIJA, SREBRENKA NEJEDLI, VELIMIR SUŠIĆ*, EMIL GJURČEVIĆ**, ŽELJKO MIKULEC***, BRANIMIR MIOKOVIĆ****, ZLATKO JANJEČIĆ*****

Department of Anatomy, Histology and Embriology, *Department of Animal Breeding and Husbandry, **Department of Biology and Pathology of Fish and Bees, ***Department of Animal Nutrition, ****Department of Foodstuffs and Technology, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia
*****Department of Animal Nutrition, Faculty of Agriculture, University of Zagreb, Svetošimunska 25, 10000 Zagreb, Croatia

Gjurčević Kantura V., Zobundžija M., Nejedli S., Sušić V., Gjurčević E., Mikulec Ž., Mioković B., Janječić Z.

Histomorphological characteristics of some muscles of Lika's Pramenka breed lambs in comparison with crossbreeds of Istrian Pramenka breed lambs

Summary

Samples of the m. triceps brachii (caput longum), m. longissimus lumborum, m. psoas major, m. gluteobiceps, m. semitendinosus, m. semimembranosus and m. quadriceps femoris (m. rectus femoris) from 5 Pramenka breed male lambs aged 3 months, weighing 20-26 kg, were obtained immediately upon slaughter and dressing, frozen in liquid nitrogen, and cut on a Cryocut into 10-µm slices stained with hematoxylin-eosin. The diameter of 4456 muscle fibers was measured on microscopic images taken on a Nikon microscope at X50 magnification. Data were presented in tables. The muscle fiber diameter ranged from 10 to 50 µm and for m. psoas major, from 10 to 45 µm. The above listed muscle samples yielded the following mean muscle fiber diameters: 22.24, 23.85, 24.70, 25.38, 24.87, 22.26 and 25.85 µm, respectively. Small diameter fibers (> 30 µm) accounted for 64.88%-91.33%, and medium diameter fibers (31-50 µm) for 7.81%-35.12% of all fibers. There were no large diameter fibers (< 50 µm). The muscle fiber surface was predominated by small diameter fibers (48.23%--80.70%), with the exception of m. triceps brachii (caput longum), where medium diameter fibers constituted up to 51.77% of the muscle fibers. Medium diameter fibers accounted for 19.30%-51.77% of the muscle fiber surface. A comparison with the previously reported observations in local Istrian Pramenka crossbreeds with Sardinian, East Friesian and domestic German Merino-Würtemberg rams revealed that the crossbreeds have all the muscular parameters considerably greater than purebred Pramenka lambs. Distribution frequency indicated the muscle fiber diameter to show a shift to the left, i.e. toward fibers of a smaller diameter in m. psoas major and m. semimembranosus, and a shift to the right, i.e. to fibers of greater diameter in m. triceps brachii (caput longum).

Keywords: lambs, Pramenka breed, muscle fiber diameter, distribution frequency

The effects of crossbreeding of local Istrian Pramenka breed sheep with Sardinian, East Friesian and German Merino-Würtemberg rams on the parameters of lamb growth, feed consumption, dressing percentage and meatiness upon slaughter at the age 71 days were investigated in a number of studies (5-7). These studies were focused on differences in the histometabolic and histomorphological properties of some muscles of the forelimb, spine and hindlimb, in order to assess the effect of the Sardinian, East Friesian and German Merino-Würtemberg genetic input on the following muscles: *m. triceps brachii* (*caput longum*), *m. longissimus lumborum*, *m. psoas major*, *m. gluteobiceps*, *m. semitendinosus*, *m. semimembranosus* and *m. quadriceps femoris* (*m. rectus femoris*) (3, 4). The number, type and diameter (minimal, maximal and mean), fiber classification into small diameter (10-30 µm), medium diameter (31-50 µm) and large dia-

meter (51-90 μm) fibers, and their percentage in the total number of measured fibers were determined in the study muscles. The improvement of the local Istrian Pramenka breed was found to have favorably influenced the parameters of lamb growth, feed consumption, dressing percentaage and meatiness (7). Histomorphological analysis of the study muscles pointed to differences in the number and size of muscle fibers, as well as in the percentage of small, medium and large diameter fibers among the three crossbreed groups (4). The muscle fiber diameter (mean) of *m. triceps brachii* (*caput longum*) was 10-75 (37.88), 25-80 (42.65) and 10-55 (30.17) μm in crossbreeds with Sardinian, East Friesian and German Merino-Würtemberg rams, respectively. The respective figures for *m. longissimus lumborum* were 12.5-90 (38.63), 15-90 (35.77) and 10-52.50 (33.88) μm; *m. psoas major* 10-65 (30.30), 10-65 (34.19) and 10-55 (33.92) μm; *m. gluteobiceps* 10-75 (37.88), 25-80 (42.65) and 10-55 (30.17) μm; *m. semitendinosus* 10-85 (49.25), 15-85 (47.56) and 10-75 (38.16) μm; *m. semimembranosus* 10-70 (45.60), 17.52-62.50 (38.78) and 20-70 (36.42) μm; and *m. quadriceps femoris* (*m. rectus femoris*) 10-90 (52.36), 17.50-60 (37.64) and 15-60 (38.23) μm. The distribution frequency of muscle fiber diameters revealed the highest number of muscle fibers in East Friesian crossbreds to show a shift to the left, i.e. between small and medium diameter fibers, except for *m. psoas major*. In Sardinian crossbreds, the majority of muscle fibers showed a shift to the right, toward large diameter fibers, with the exception of *m. psoas major* and *m. gluteobiceps*. According to the authors (4), the use of distribution frequency of muscle fiber diameters (1) proved helpful in the monitoring and evaluation of zootechnological procedures such as meatiness analysis as well as in the analysis of the same and different muscles in the same or different animal species (3, 4), or in the analysis of complex muscles in the same animal species (1, 2, 10) and in humans (9).

The local Pramenka sheep breed includes several strains adaptable according to geographical-climatic regions of Croatia, whereby each strain can be or has already been used for breed improvement. As data on the histomorphological muscle characteristics of Pramenka lambs are lacking, the aim of the present study was to analyze the above listed skeletal muscles in purebred Pramenka lambs before crossbreeding. Muscles from lambs of the Lička Pramenka breed were initially chosen for comparison to the Istrian Pramenka crossbreds with Sardinian, East Friesian and German Merino-Würtemberg rams (4) according to histomorphological properties.

Material and methods

The study included 5 male lambs of the Lika's Pramenka breed, aged 3 months, weighing 20, 23, 23, 25 and 26 kg. The lambs were kept on pasture with additional age-adjusted feed. Like our previous study, the following muscles of the forelimb, spine and hindlimb were studied: *m. triceps brachii* (*caput longum*), *m. longissimus lumborum*, *m. psoas major*, *m. gluteobiceps*, *m. semitendinosus*, *m. semimembranosus* and *m. quadriceps femoris* (*m. rectus femoris*). Muscle samples were obtained immediately upon slaughter and dressing from the following sites: *m. triceps brachii* (*caput longum*) and *m. quadriceps femoris* (*m. rectus femoris*) from the central part of the muscles; *m. longissimus lumborum* and *m. psoas major* at the level of the third lumbar vertebra; and *m. gluteobiceps*, *m. semitendinosus* and *m. semimembranosus* at the level of the hip joint. Muscle samples of 1 × 1 cm in size were frozen in liquid nitrogen, and cut on a Cryo-cut into 10 μm slices, which were stained with hematoxylin and eosin (8) for muscle structure analysis. Images of these muscle slides were taken on a Nikon microscope at X50 magnification. On these images, muscle fibers were counted and their diameter measured in a total of 4456 fibers. The results thus obtained were presented in tables, pooled and separate for each study muscle, and by the use of distribution frequency of fiber diameters in 5 μm categories (1). In addition, the number of small and medium diameter fibers, and their percentage in the total muscle fiber surface are presented.

Results and discussion

The findings obtained showed the study muscles to differ according to fiber number and diameter. Differences were also recorded in the number of muscle fibers and their diameter in the same muscle from various lambs. The variation in the number and diameter of muscle fibers entailed differences in the size of the relative muscle surface.

The diameter of muscle fibers ranged from 10-50 μm, with the exception of *m. psoas major*, where the greatest fiber diameter of 45 μm was recorded, with no fibers of 50 μm in diameter. Out of 4456 muscle fibers counted, *m. psoas major* constituted the highest proportion (fig. 1), immediately followed by *m. longissimus lumborum* and *m. gluteobiceps*, then *m. semitendinosus* and *m. triceps brachii* (*caput longum*),

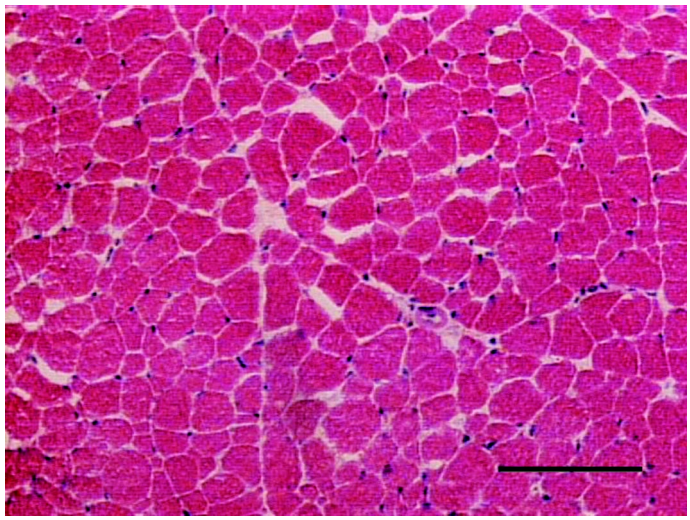

Fig. 1. *M. psoas major*; lamb; male; Lika's Pramenka breed; Hematoxylin-eosin (scale bar = 100 μm)

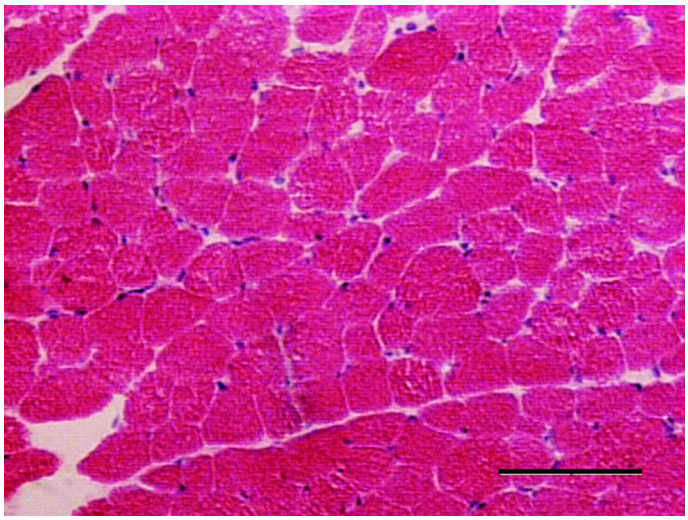

Fig. 2. *M. quadriceps-femoris* (*M. rectus femoris*); lamb, male, Lika's Pramenka breed; Hematoxylin-eosin (scale bar = 100 µm)

whereas *m. semimembranosus* and *m. quadriceps femoris* (*m. rectus femoris*) had the lowest number of fibers in the microscope field (fig. 2). Expressed in percentages, *m. triceps brachii* (*caput longum*), *m. longissimus lumborum*, *m. psoas major*, *m. gluteobiceps*, *m. semitendinosus*, *m. semimembranosus* and *m. quadriceps femoris* (*m. rectus femoris*) accounted for 11.69%, 16.09%, 22.42%, 14.72%, 11.13%, 13.22% and 10.73% of the fibers, respectively (tab. 1).

Classification of muscle fibers according to their diameter into fibers of small diameter (10-30 µm), medium diameter (31-50 µm) and large diameter (51--90 µm) fibers revealed that fibers of small diameter are predominant (tab. 2). The proportion of small diameter fibers ranged from 64.88% in *m. triceps brachii* (*caput longum*) through 92.19% in *m. psoas major*, with other study muscles falling in between: *m. quadriceps femoris* (*m. rectus femoris*) 78.82%, *m. semitendinosus* 82.00%, *m. gluteobiceps* 82.16%, *m. longissimus lumborum* 85.50%, and *m. semimembranosus* 91.33%. The proportion of medium diameter fibers was considerably lower, ranging from 7.81% in *m. psoas major* through 35.12% in *m. triceps brachii* (*caput*

Tab. 1. Comparative presentation of number and percentage of muscle fibers

Diameter µm	Muscle													
	TB		LL		PM		GB		ST		SM		QF	
	number	%	number	%	number	%	number	%	number	%	number	%	number	%
10	2	0.38	39	5.44	37	3.70	22	3.36	29	4.92	25	5.04	7	1.46
15	31	5.95	96	13.39	133	13.31	68	10.37	51	8.66	90	18.15	31	6.49
20	46	8.83	156	21.76	329	32.93	167	25.46	144	24.45	153	30.85	142	29.71
25	128	24.57	188	26.22	269	26.92	182	27.74	136	23.09	113	22.78	118	24.69
30	131	25.14	134	18.69	143	14.31	100	15.24	123	20.88	72	14.52	81	16.95
35	154	29.56	81	11.29	71	7.11	92	14.02	80	13.58	26	5.24	71	14.85
40	21	4.03	19	2.65	15	1.50	16	2.44	18	3.06	12	2.42	18	3.77
45	5	0.96	3	0.42	2	0.20	8	1.22	7	1.19	4	0.80	8	1.67
50	3	0.58	1	0.14	–	–	1	0.15	1	0.17	1	0.20	2	0.42
Total	521	100	717	100	999	100	656	100	589	100	496	100	478	100

Explanations: TB = *M. triceps brachii* (*caput longum*), LL = *M. longissimus lumborum*, PM = *M. psoas major*, GB = *M. gluteobiceps*, ST = *M. semitendinosus*, SM = *M. semimembranosus*, QF = *M. quadriceps femoris* (*m. rectus femoris*)

Tab. 2. Presentation of muscle fibers with respect the number and diameter

Muscle	Fibers according to the diameter								Fibre diameters µm		
	Measured fibers		Small 10-30 µm		Medium 31-50 µm		Big 50-90 µm		Mean	Minimal	Maximal
	number	%	number	%	number	%	number	%			
M. triceps brachii	521	11.69	238	64.88	183	35.12	–	–	22.24	10	50
M. longissimus lumborum	717	16.09	613	85.50	104	14.50	–	–	23.85	10	50
M. psoas major	999	22.42	921	92.19	78	7.81	–	–	24.70	10	45
M. gluteobiceps	656	14.72	539	82.16	117	17.84	–	–	25.38	10	50
M. semitendinosus	589	13.22	483	82.00	106	18.00	–	–	24.87	10	50
M. semimembranosus	496	11.13	453	91.33	43	8.67	–	–	22.26	10	50
M. quadriceps femoris	478	10.73	372	78.82	106	22.18	–	–	25.85	10	50
Total	4456	100	3719	100	737	100	–	–			

Tab. 3. Procentual presentation of muscle fiber surface with respect to diameter

Muscle	Number	Total surface/ μm²	Fibers with respect to diameter					
			Small 10-30 μm			Medium 31-50 μm		
			number	surface/μm²	%	number	surface/μm²	%
M. triceps brachii	521	36372.974	338	17542.787	48.23	183	18830.187	51.77
M. longissimus lumborum	717	36439.689	613	25591.000	70.23	104	10898.689	29.77
M. psoas major	999	45300.386	921	36270.924	80.07	78	9029.625	19.93
M. gluteobiceps	656	34032.105	539	22611.925	66.44	117	11420.180	33.56
M. semitendinosus	589	25212.774	483	13950.987	55.33	106	11262.787	44.67
M. semimembranosus	496	22064.562	453	17225.937	78.07	43	4839.525	21.93
M. quadriceps femoris	478	27325.849	372	16573.312	60.65	106	10752.537	39.35
Total	4456		3719		100	737		100

longum). Other muscle types showed the following proportion of these fibers: *m. quadriceps femoris* (*m. rectus femoris*) 22.18%, *m. semitendinosus* 18.00%, *m. gluteobiceps* 17.84%, *m. longissimus lumborum* 14.50%, and *m. semimembranosus* 8.67%. Muscle fibers of a large diameter were not found at all (tab. 2).

Small diameter fibers accounted for the major part of the muscle fiber surface, their proportion ranging from 55.33% through 80.07% (tab. 3), with the exception of *m. triceps brachii* (*caput longum*). In *m. triceps brachii* (*caput longum*), small diameter and medium diameter fibers accounted for 48.23% and 51.77% of the overall fiber surface, respectively. The percentage of medium diameter fibers in the overall fiber surface for other muscles was as follows: *m. psoas major* 19.93%, *m. semimembranosus* 21.93%, *m. longissimus lumborum* 29.77%, *m. gluteobiceps* 33.56%, *m. quadriceps femoris* (*m. rectus femoris*) 39.35%, and *m. semitendinosus* 44.67%.

The distribution frequency of muscle fiber diameter at 5-μm categories indicated 25-35 μm fibers to prevail in *m. triceps brachii* (*caput longum*); 20-30 μm fibers in *m. longissimus lumborum* and *m. semitendinosus*; and 20-25 μm fibers in *m. psoas major*, *m. gluteobiceps*, *m. semimembranosus* and *m. quadriceps femoris* (*m. rectus femoris*) (tab. 1).

Analysis of the histomorphological characteristics of the study muscles from Lika's Pramenka lambs showed that investigated muscles vary within individual and among different animals. Differences were quite high relative to the total number of muscle fibers *per* microscope field according to both the number of small and medium diameter fibers, and the surface covered by these fibers. Differences were also observed in the mean diameter size. The least difference was recorded in the range of diameter size. In all study muscles, the smallest fiber diameter of 10 μm and largest diameter of 50 μm were measured, except for *m. psoas major*, where the largest fiber diameter was 45 μm. Comparison of the muscle fiber diameters measured in this study with data from the study on the effect of Istrian Pramenka crossbreeding with the Sardinian, East Friesian and German Merino-Würtemberg rams (4) reveals the fiber diameter of the study muscles from crossbred lambs aged 3 months and weighing 21.74-25.43 kg to exceed the respective figures recorded in age- and weight-matched Lika's Pramenka lambs. The muscle fiber diameter in the crossbred lambs ranged from 10 to up to 90 μm, whereas in the present study it was only 10-50 μm, and 10-45 μm for *m. psoas major*. The study muscles from crossbred lambs were predominated by medium diameter fibers (49%-77%), except for *m. semimembranosus* and *m. quadriceps femoris* (*m. rectus femoris*) in crossbreds with Sardinian rams, where medium diameter fibers were not the predominant type of fiber and accounted for 42% and 26.40% of total muscle fiber, respectively; however, the number of large diameter fibers exceeded the number of medium diameter fibers in both cases. In the present study, the proportion of medium diameter fibers was considerably lower than that of small diameter fibers, whereas large diameter fibers were not observed at all. Medium diameter fibers accounted for 7.81% to 35.12% of all fibers in the study muscles. The percentage of medium diameter fibers in *m. semimembranosus* was 8.67%, which would only to a certain extent correspond to the lower percentage of this fiber type in crossbreeds with Sardinian rams (4). This, however, does not apply to *m. quadriceps femoris* (*m. rectus femoris*), which was found to contain 22.18% of medium diameter fibers, i.e. a high percentage relative to other muscles. The respective muscles from Sardinian crossbreeds had 26.40% of medium diameter fibers, i.e. an extremely low percentage relative to other muscles. In the present study, the lowest proportion of medium diameter fibers (7.81%) was found in *m. psoas major*, whereas the same muscle from Sardinian, East Friesian and German Merino-Würtemberg crossbreds showed 48.60%, 58.10% and 61.70% of this fiber type, respectively (4).

In the present study, the percentage of small diameter fibers ranged from 64.88% through 92.11% and accounted for the majority of all muscle fibers. In Istrian

Pramenka crossbreeds with Sardinian, East Friesian and German Merino-Würtemberg rams, the lowest percentage of small diameter fibers was recorded in *m. gluteobiceps* of Sardinian crossbreds (5.30%), and *m. semitendinosus* and *m. triceps brachii* (*caput longum*) of Sardinian and German Merino-Würtemberg crossbreeds (6.80% and 6.40%; and 4.00% and 1.50%, respectively). The highest percentage of small diameter fibers was found in *m. gluteobiceps* and *m. psoas major* of Sardinian crossbreeds (45.60% and 45.40%, respectively).

The mean diameter of muscle fibers ranged from 22.24 to 25.80 μm, and was smallest in *m. triceps brachii* (*caput longum*) (22.24 μm) and *m. semimembranosus* (22.26 μm). The largest fiber diameter was recorded in *m. gluteobiceps* (25.38 μm) and *m. quadriceps femoris* (*m. rectus femoris*) (25.85 μm). In the muscles from crossbred animals, the mean muscle fiber diameter ranged from 30.17 through 52.36 μm; it was largest in *m. quadriceps femoris* (*m. rectus femoris*) of Sardinian crossbreds (52.36 μm), and smallest in *m. gluteobiceps* of East Friesian crossbreeds (30.17 μm) and *m. psoas major* of Sardinian crossbreds (30.30 μm).

The use of distribution frequency of muscle fiber diameter (1) showed a somewhat different pattern. Fibers of 20-30 μm in diameter predominated in *m. longissimus lumborum* and *m. semitendinosus*, supporting movement of moderate intensity. Fibers of 20-25 μm in diameter prevailed in *m. psoas major* (in particular), *m. gluteobiceps*, *m. semimembranosus* and *m. quadriceps femoris* (*m. rectus femoris*), and those of 25-35 μm in diameter in *m. triceps brachii* (*caput longum*). Fibers of a larger diameter were present in lower proportion, especially in *m. semimembranosus* and *m. psoas major*. Such fibers of a larger diameter are able to function at a higher load. Considering the higher proportion of particular fiber types, distribution frequency revealed the muscle fibers of *m. psoas major* and *m. semimembranosus* to show a shift to the left, i.e. toward smaller diameter fibers, and those of *m. triceps brachii* (*caput longum*) a shift to the right, i.e. toward greater diameter fibers.

The presentation of the muscle surface covered by small and medium diameter fibers showed the surface percentage of small diameter fibers to exceed the surface percentage of medium diameter fibers in all study muscles except for *m. triceps brachii* (*caput longum*). Thus, *m. psoas major* and *m. semimembranosus*, indicated by diameter distribution frequency to be shifting to the left, had the smallest surface covered by medium diameter fibers (19.93% and 21.93%, respectively), whereas *m. triceps brachii* (*caput longum*), indicated by diameter distribution frequency to show a shift to the right, had the largest surface covered by medium diameter fibers (51.77%). This is quite conceivable because a higher proportion of larger diameter fibers will have a greater area.

The indicators examined in this study may prove useful in assessing the effects of crossbreeding local primitive sheep breeds with purebred rams of milky, meaty or combined properties and they could indicate the meatiness increase in crossbreeds. The present study also demonstrated the use of distribution frequency of muscle fiber diameters to be helpful in muscle studies.

References

1. *Bego U.*: Frequency distribution of muscle fibre diameters on the pigeon's pectoral muscle and a new fibre classification. Period. Biol. 1994, 96, 53-56.
2. *Bego U., Mitin V., Zobundžija M.*: New approach to the functional classification of horse diaphragma muscle fibres. Praxis Veter. 1966, 44, 159-175.
3. *Gjurčević-Kantura V., Mikulec K., Zobundžija M., Sušić V., Mihelić D., Mikulec Ž., Vučemilo M.*: Histometabolic properties of some skeletal muscles in crossbreed lambs of croatian dairy sheep. Czech J. Anim. Sci. 1999, 44, 463-469.
4. *Gjurčević-Kantura V., Zobundžija M., Mikulec K., Sušić V., Mihelić D., Šerman V., Mikulec Ž., Pipić R., Vučemilo M.*: The analysis of some skeletal muscles in lambs of different genotype by the distribution frequency of muscle fibres diameter. Czech J. Anim. Sci. 2000, 45, 257-262.
5. *Mikulec K., Šerman V., Sušić V.*: Revival and improvement of sheep and goat production in the Republic of Croatia. Krmiva 1992, 34, 155-160.
6. *Mikulec K., Sušić V., Pipić R.*: Breeding objectives and breed important for inproving sheep breeding in the Republic of Croatia. Vet. Stanica 1996, 77, 9-16.
7. *Mikulec K., Sušić V., Šerman V., Mikulec Ž., Pipić R., Balenović T., Matičić D.*: Lamb fattening and carcas characteristics of croatian crossbred dairy sheep. Vet. Med. Czech, 1997, 42, 337-342.
8. *Romeis B.*: Mikroskopische Technik. Oldenburg Verlag, München und Wien 1968.
9. *Valentić-Peruzović M., Bego U., Bobinac D.*: Influence of speech and bite on ENG and morphohistochemical characteristics of masseter and temporalis, [in:] Proc. 10th Int. Symp. Biomedical Engeneering 94, Zagreb 1994, p. 138--142.
10. *Zobundžija M., Bego U., Mitin V., Gjurečvić-Kantura V.*: Application of the distribution frequency of muscle fibre diameter of muscle tissue in morpho-functional analysis of the diaphragm in fattened bulls. Praxis Veter. 1998, 46, 195-202.

Author's address: Vesna Gjurčević Kantura PhD, Department of Anatomy, Histology and Embriology, Faculty of Veterinary Medicine University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia; e-mail: kantura@vef.hr