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POSTMORTEM STATE OF PORCINE MUSCLE TISSUE DEPENDING ON PRE-SLAUGHTER FASTING TIME

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Abstract

Changes in the muscle tissue microstructure lead to changes in meat quality. One of the causes of the myopathy development is animal stress. Pigs experience the strongest stress during pre-slaughter holding. The study of the postmortem meat structure depending on fasting time is a topical task. The objects of the research were samples of m. L. dorsi obtained after slaughter from pigs that differed in fasting time: 4 (group 1), 8 (group 2), 10 (group 3), 16 (group 4) and 18 (group 5) hours (N = 20, n = 4). Investigation of the microstructure and morphometric measurements were carried out on preparations stained with hematoxylin and eosin. Myopathic changes in muscle tissue were assessed using a semi-quantitative method developed earlier. All studied samples were characterized by the uniform condition of muscle tissue. Statistically significant differences between individual groups were observed regarding the number and area of giant fibers, sarcomere length, diameter of muscle fibers and proportion of muscle fibers, which diameter was lower or higher by 1/3 than the mean fiber diameter. An increase in the pre-slaughter holding time reduced the number and area of giant fibers (r = –0.8437 and –0.5796, respectively), as well as the diameter of "normal" fibers (r = –0.5337), which positively influenced pork quality. Groups 1, 2 and 3 were characterized by the presence of signs of moderate and pronounced myopathy. Only one carcass with pronounced myopathic signs was revealed in each of groups 4 and 5. In group 4, one carcass did not have signs of myopathy. Pre-slaughter holding during 4, 8 and 10 hours led to deterioration of pork quality. The recommended fasting time is 16 hours.

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Introduction

From the end of the 20th century, the mass fraction of muscle tissue in porcine carcasses has become an extremely important indicator demonstrating successes of modern pig husbandry. At the same time, the topicality of the problem of pork quality and its technological suitability has grown. The negative correlation between the quantity of muscle tissue in a carcass and pork quality is becoming more and more evident [1]. The genetic progress increased the burden on fast-growing slaughter animals, led to morphological and biochemical modifications of muscle tissue and, as a consequence, to deterioration of consumer properties of meat [2].

In addition to the scientific value, the study of the muscle tissue microstructure is of great practical importance. Muscle fibers are key components of skeletal muscles, which characteristics significantly influence meat quality [3,4]. Histological changes in muscle tissue lead to changes in meat quality [5,6].

Joint histological and sensory studies show that even changes in pork palatability can be explained by characteristics of muscle fibers [7,8]. It is fairly considered that the knowledge of the muscle tissue microstructure with the use of simple methods of its differentiation can be a crucial element of adequate and objective assessment of meat quality [1].

Muscle tissue lesions, in which pathological changes in muscle fibers are observed ranging from degenerative changes to postmortem development of hypertonus giant fibers, are assigned to the main signs of myopathy (Greek: mys, my[os] muscle + pathos: suffering, disease) [2]. All animal species and even insects are prone to myopathy. The causes of myopathy can be different, the main among them are genetics, nutrition and stress. An effect of stress on the development of myopathic changes in porcine muscle tissue is well known [6,9].

Pigs experience the longest and multi-factor stress during pre-slaughter holding (fasting). Fasting is an obligatory measure before slaughtering pigs, which ensures:

- pork safety (visual contamination and microbial contamination of carcasses are reduced) [10];
- pork quality (allow obtaining chilled meat with the optimal pH value and improved technological characteristics) [11,12].

In terms of animal welfare, it reduces the proportion of animals died during transportation [13,14] and stunned with incorrect result [15], and increases heat resistance in pigs [16,17,18].

Pre-slaughter keeping, especially, accompanied by extreme overcrowding, water deficiency, mixing animals from different groups, is a well-known stress factor for pigs [19]. A high level of stress causes behavioral deviations and reduces meat quality. Postmortem redox processes in such pork are characterized by the development of porcine stress syndrome and production of meat with signs of myopathy due to enhanced and prolonged postmortem glycolysis [20]. This meat is characterized by histopathological deviations in muscles and appearance of destructive changes (sarcolemma disruptions), alterations of muscle fiber shape, appearance of atrophied fibers as well as hypertrophied and giant fibers [21].

In our view, it is of scientific and practical interest to study an effect of fasting time on a muscle tissue condition and a degree of the development of myopathic changes.

Previously, the authors developed approaches to classification of porcine carcasses by a degree of manifestation of myopathic changes in muscle tissues into three groups: without myopathy, with signs of moderately pronounced myopathy and pronounced myopathy [22].

The aim of this work was to study a postmortem condition of muscle tissue depending on duration of pig's fasting on the basis of two approaches: analysis of the mean values of morphometric characteristics and scoring of a degree of manifestation of myopathic signs.

Objects and methods

Control slaughter of pigs (n = 100) with an average life weight of 118.1 ± 5.4 kg was carried out in an industrial enterprise slaughtering pigs in an amount of 800 heads per day. Animals for control slaughter were randomly chosen and divided into groups of 20 animals from five different sets differed by fasting time: 4, 8, 10, 16 and 18 hours for groups 1, 2, 3, 4 and 5, respectively.

Animals were slaughtered in the same conditions using gas stunning (CO_2 concentration 88%, exposure time 120 s). Hot carcasses were sent to one-stage chilling at a temperature of 2 ± 2 °C. After 24 hours, four carcasses were randomly selected from each group (N = 20, average weight of selected carcasses was 87.8 ± 2.8 kg) for histological investigations.

For microstructural analysis, samples with a size of 3 × 3 × 3 cm were taken from *m. Longissimus dorsi* (*m. L. dorsi*). Samples were fixed in 10% neutral buffered formalin for 72 hours at room temperature. For the following study, two pieces (1.5×1.5×0.5 cm) with longitudinal and cross orientation of muscle fibers were taken from each sample. The pieces were washed with cold running water for four hours. Then, they were embedded in gelatin in an ascending concentration (12.5%, 25%) at a temperature of 37 °C for 8 hours each.

Serial sections with a thickness of 16 μm were made on the cryostat «MIKROM–HM525» (Thermo Scientific, USA). Three sections were made from each piece. The obtained sections were mounted on Menzel-Glaser slides (Thermo Scientific, USA) and stained with Ehrlich's hematoxylin and 1% aqueous-alcoholic solution of eosin (BioVitrum, Russia) by the conventional method (Romeis, B., 1989). The histological preparations were studied and photographed using an Axio Imager A1 light microscope (Carl Zeiss, Germany) with the AxioCam MRc-5 camera (Carl Zeiss, Germany). The muscle tissue condition (shape of muscle fibers, condition of sarcolemma, striation) and a degree of destructive changes were analyzed.

Morphometric studies were performed using the image analysis system AxioVision 4.7.1.0 (Carl Zeiss, Germany). Packing density of muscle fibers (the number of fibers with the normal diameter (hereinafter, normal fibers)/ 1 mm^2), their diameter, sarcomere length, the number of giant fibers located on 1 cm^2, their diameter and cross-sectional area were measured in the interactive mode. For each section, no less than 100 objects were calculated. A fiber diameter was determined with an accuracy of ±1.0 μm. A sarcomere length was measured with an accuracy of ± 0.1 μm.

Myopathic changes in muscle tissue were assessed using the semi-quantitative scoring method developed jointly by the V. M. Gorbatov Federal Research Center for Food Systems and L. K. Ernst Federal Research Center for Animal Husbandry[1] (Table 1).

Statistical analysis of the experimental data was carried out using the software R (version 4.2.1). Quantitative data are presented as the arithmetic mean (Mean), standard error of the mean (SE), standard deviation (SD), minimum and maximum values (Min/Max), confidence interval (CI) и median. The normality of distribution of parameters of quantitative variables was assessed by the Kolmogorov–Smirnov test. The interrelation of the factor under study with morphometric indices of muscle tissue was determined on the sample of animals by methods of the one-way analysis of variance (ANOVA) and Dunnett's test. Differences were considered significant and the presence of a relationship between parameters was recognized at a probability level of not higher than 0.05.

Results and discussion

In our experiment, pre-slaughter holding was limited to 18 hours due to the practice of industrial enterprises and earlier obtained data showing that longer periods are not economically expedient because of the live weight loss [11]. Moreover, pigs from the same enterprise, the same feeding and age were sent to slaughter. This excluded significant deviations in animal weight (in our experiment they were not more than ±5.4 kg) and did not envisage an effect of live weight on morphometric indices [22].

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Table 1. Scheme of scoring of manifestation of myopathic signs in analysis of the muscle tissue microstructure

Indicator	Characteristics (value) of indicator/assigned points		
	Without signs of myopathy *	Moderately pronounced myopathy **	Pronounced myopathy ***
Shape of muscle fibers	Slightly wavy, tightly arranged /1	Mainly straight, tightly arranged /2	Straight, located loosely relative to each other/3
Condition of cross-striation	Clearly defined/1	Minute, located closer, smoothened, irregular/2	Minute, located closer, smoothened, irregular/2
Average length of sarcomeres, μm	From 2.0 inclusively and more/1	1.6–1.9 inclusively/2	Less than 1.6/3
Presence of destructive changes in sarcolemma	Not found/1	Individual ruptures of sarcolemma are present /2	Multiple ruptures of sarcolemma are present /3
Presence of giant fibers contraction knots), numbers/1 cm^2	Not found and/or few (up to 10) are found /1	From 10 inclusively to 30/2	30 and more/3
Average area of giant fibers on the cross section, μm^2	Up to 10,000/1	From 10,000 inclusively to 15,000/2	15,000 and more /3
Packing density of muscle fibers (number per unit of cross-sectional area), fibers/mm^2	250 and more/1	From 150 inclusively to 250/2	Up to 150/3
Proportion of muscle fibers, which diameter is lower or higher by 1/3 than the mean fiber diameter,%	Up to 7 inclusively/1	From 8 inclusively up to 30/2	30 and more/3

Notes:
*** by results of evaluation, samples that did not have a score of three points for any indicator and received no more than 12 points, inclusively, are classified as muscle tissue without signs, with destructive changes corresponding to the normal development of autolytic processes;**
**** samples that received from 13 to 16 points, inclusively, are classified as muscle tissue with moderately pronounced signs of myopathy;**
***** samples that received more than 16 points are classified as muscle tissue with pronounced signs of myopathy.**

All studied samples were characterized by the uniform condition of muscle tissue. On the cross-section, muscle fibers had the polygonal or weakly round shape. The endomysium interlayers were well pronounced; the boundaries between individual muscle fibers were established without particular difficulties. Giant fibers were characterized by a round-oval shape and large diameter.

In the longitudinal section, the main mass of muscle fibers was characterized by the well-defined cross-striation and straightened shape. Individual wavy fibers with longitudinal striation were found, which suggested the presence of contraction zones.

The nuclei in muscle fibers were well stained, had the oval shape and were located directly under the sarcolemma.

The perimysial connective tissue interlayers were wavy, tightly adjacent to bundles of muscle fibers. The nuclei in the connective tissue interlayers were clearly defined on the histological slides. Individual adipocytes or small groups of adipocytes with the typical histological structure were revealed between the bundles of muscle fibers in the areas of perimysium.

In the normal muscle fibers, individual cross micro-ruptures and sarcolemma damage were observed; destruction of myofibrils and ruptures were not found. In the knots of hyper-contraction (giant fibers on the longitudinal section), destruction of sarcomeres and the presence of cracks and ruptures of fibers were noticed.

Results of morphometry of samples are given in Table 2.

The number and size of muscle fibers to a large degree are associated with meat quality [23,24]. Muscle tissue with lower diameter and higher density of muscle fibers is linked with higher meat quality than muscle tissue with higher fiber area and lower fiber density [3].

By the mean values of morphometric indicators (Table 2), group 4 had several advantages, such as:

- normal muscle fibers were characterized by the lowest mean values of the fiber diameter and sarcomere length, as well as the number and area of giant fibers per 1 cm^2;
- muscle tissue was characterized by the highest packing density of muscle fibers;
- proportion of muscle fibers, which diameter was higher or lower by 1/3 than the mean value, was lower than in groups 3 and 5 but comparable with the value of this indicator in group 1;

By the mean values of morphometric indicators, group 5 was close to group 4, but significantly differed from it by a higher sarcomere length and higher values of area of giant fibers.

According to the analysis of the morphometric indicators, short period of pre-slaughter holding led to an increased number of giant fibers per units of section area and an increase in their area, which invariably have to be linked with lower meat quality [23,24].

Statistically significant differences between individual groups were observed by the following indicators: number and area of giant fibers on the cross section, sarcomere length, muscle fiber diameter and proportion of muscle fibers, which diameter was lower or higher by 1/3 than the mean fiber diameter. In terms of packing density of muscle fibers, no significant differences were found between groups.

Assessment of correlation between the studied morphometric indicators and the duration of pre-slaughter holding (Table 3) shows that several indicators have a tendency towards changes with an increase in the duration of pre-slaughter holding:

Table 2. Morphometric indicators of muscle tissue in ***m. L. dorsi*** samples (N = 20, n = 4)

Indicator		Value of indicator for groups				
		1 (4 h)[a]	2 (8 h)[b]	3 (10 h)[c]	4 (16 h)[d]	5 (18 h)[f]
Diameter of muscle fibers, μm	k	600	600	600	600	600
	Mean (SD)	67.33 ± 17.90	68.81 ± 24.13[d]	69.70 ± 22.29[df]	65.74 ± 17.56[b]	66.67 ± 22.87[c]
	Min…Max	18.07…116.42	17.01…180.46	21.88…133.50	25.53…110.45	17.01…140.88
	Median	67.80	67.19	69.17	66.09	67.91
	SE	0.73	0.98	0.91	0.72	0.93
	95% CI	1.43	1.93	1.79	1.41	1.83
Sarcomere length, μm	k	40	40	40	40	40
	Mean (SD)	17.00 ± 0.97[d]	17.14 ± 1.49[d]	16.67 ± 1.04[f]	16.50 ± 1.00[abf]	17.44 ± 0.86[cd]
	Min…Max	15.40…18.71	13.84…19.98	14.80…19.13	14.59…18.43	15.18…18.81
	Median	16.86	17.31	16.85	16.30	17.42
	SE	0.15	0.24	0.16	0.16	0.14
	95% CI	0.31	0.48	0.33	0.32	0.28
Packing density of muscle fibers (number per unit of cross-sectional area), fibers/mm²	k	12	12	12	12	12
	Mean (SD)	154.17 ± 37.97	161.33 ± 34.60	150.17 ± 19.71	160.83 ± 32.38	158.33 ± 34.10
	Min…Max	104…225	107…207	129…193	102…198	98…198
	Median	146.0	173.5	147.0	167.0	169.5
	SE	10.96	9.99	5.69	9.35	9.84
	95% CI	24.13	21.98	12.52	20.57	21.67
Number of giant fibers, fibers /1 cm²	k	12	12	12	12	12
	Mean (SD)	13.83 ± 6.95[bdf]	9.08 ± 2.15[ad]	11.50 ± 7.55[d]	4.33 ± 2.67[abc]	7.42 ± 6.07[a]
	Min…Max	6…23	6…11	1…23	0…8	0…16
	Median	13.5	10.0	11.0	5.0	7.0
	SE	2.01	0.62	2.18	0.77	1.75
	95% CI	4.42	1.37	4.80	1.70	3.85
Area of giant fibers on the cross section, μm²	k	166	109	138	52	89
	Mean (SD)	15638.2 ± 2877.7[bcd]	18746.7 ± 4329.1[acdf]	16428.2 ± 3310.6[abdf]	12103.6 ± 2869.02[abcf]	15008.1 ± 2409.8[bcd]
	Min…Max	9057.0…26045.4	12441.8…33103.6	10007.00…28412.7	7969.4…18289.1	10656.4…24577.6
	Median	15648,3	17589,2	16001,9	11338,5	14660,3
	SE	223.4	414.6	281.8	397.9	255.4
	95% CI	441.0	821.9	557.3	798.7	507.6
Proportion of muscle fibers, which diameter was lower or higher by 1/3 than mean fiber diameter, %	k	600	600	600	600	600
	Mean (SD)	23.33 ± 5.52[c]	31.83 ± 12.43	39.00 ± 6.66[ad]	25.33 ± 7.79[c]	32.50 ± 14.06
	Min…Max	18.67…31.33	22.00…50.00	34.00…48.67	17.33…36.00	16.00…50.00
	Median	21.67	27.67	36.67	24.00	32.00
	SE	2.76	6.21	3.33	3.90	7.03
	95% CI	8.79	19.77	10.59	12.40	22.38

Notes:
k — number of observations. Mean — arithmetic mean. SD — standard deviation. Min — minimum value. Max — maximum value. SE — standard error of the mean. CI — confidence interval.
Letters denote values that have statistically significant ($p < 0.05$) differences from the similar value of the corresponding groups.

- packing density of muscle fibers increased, which apparently was a result of the moisture loss by animals (although watering was stopped only three hours before slaughter, not all animals drank water and had a free access to drinkers; apparently, the existing norms of area (0.6 m²/head) are not sufficient for this);
- diameter of "normal" muscle fibers decreased, which corresponded to an increase in their density; these indicators were also characterized by quite a strong correlation (r = –0.5498);
- number and area of giant fibers decreased; these indicators were also interrelated (r = 0.5615).

Analysis of other indicators allowed revealing positive correlations between morphometric indicators.

Table 3. Correlation coefficients (r)

	x_1	x_2	x_3	x_4	x_5	x_6	x_7
x_1	1.0000	-0.5337	0.0626	0.3858	-0.8437	-0.5796	0.1938
x_2		1.0000	0.0040	-0.5498	0.6029	0.8170	0.7208
x_3			1.0000	0.2146	0.1614	0.4586	0.0848
x_4				1.0000	-0.7254	-0.1386	-0.4013
x_5					1.0000	0.5615	0.0679
x_6						1.0000	0.4557
x_7							1.000

Where: x_1 — duration of pre-slaughter holding; x_2 — diameter of muscle fibers; x_3 — sarcomere length; x_4 — packing density of muscle fibers; x_5 — number of giant fibers; x_6 — area of giant fibers on the cross section; x_7 — proportion of muscle fibers, which diameter was lower or higher by 1/3 than the mean fiber diameter.

For example, a positive correlation (r = 0.6029 and 0.8170, respectively) was observed between the diameter of "normal" muscle fibers and the number and area of giant fibers. In other words, an increase in the diameter of "normal" muscle fibers led to a growth in the number and area of giant fibers.

The same relationship was found for a diameter of muscle fibers and the proportion of muscle fibers, which diameter was lower or higher by 1/3 than the mean fiber diameter/"non-standard" size/ (r = 0.7208), that is, with an increase in a diameter of "normal" muscle fibers, the proportion of normal fibers differed to a large degree from the mean diameter increased. To put it otherwise, the heterogeneity of muscle fibers in terms of diameter grew.

In addition, the positive correlation was observed between the sarcomere length and the area of giant fibers (r = 0.4586), as well as the area of giant fibers and proportion of fibers of the "non-standard" size (r = 0.4557).

The negative correlation linked the packing density of muscle fibers with the number of giant fibers and proportion of muscle fibers, which diameter differed by less or more than 1/3 from the mean diameter (r = –0.7254 and –0.4013, respectively). This did not correspond to the concept that giant fibers appear as a result of supercontraction of individual muscle fibers [25,26,27]. Our data more likely confirm a more recent hypothesis that the development of giant fibers occurs from muscle fibers exhausted before slaughter due to animal stress [23,27]. Such fibers have changed metabolism and can experience the very quick onset of rigor mortis, while adjacent fibers continue to be in the relaxed state. In this connection, the negative correlation between the aforementioned indicators can characterize the disproportion in the development of post-mortem changes in muscle fibers.

Therefore, the performed analysis of the mean values of morphometric indicators in the samples taken from 20 carcasses shows that the microstructure of chilled porcine muscle tissue depends on the duration of pre-slaughter holding of pigs. Moreover, an increase in pre-slaughter holding time reduced the number and area of giant fibers as well as a diameter of "normal" fibers. These changes, in turn, influenced practically all morphometric indicators. With that, the sarcomere length practically did not depend on fasting time but showed the dependence on the area of giant fibers.

For transition from the quantitative values of morphometric indicators to values that characterize the qualitative condition of muscle tissue, scoring of each sample was carried out, which allowed making conclusion about the presence and degree of manifestation of myopathic changes (Table 4). For illustration, the final scores (total points and conclusions) are given in Table 5.

Table 4. Results of scoring of morphometric indicators of muscle tissue samples from the control and experimental groups (N = 20, n = 4)

Indicator	Scoring (points) for samples from groups			
	Carcass 1	Carcass 2	Carcass 3	Carcass 4
Group 1 (4 h)				
Shape of muscle fibers	1	2	3	3
Condition of cross-striation	1	1	1	1
Mean sarcomere length, μm	3	2	2	2
Presence of destructive changes in sarcolemma	2	2	2	2
Presence of giant fibers (contraction knots), fibers/1 cm^2	2	2	1	1
Average area of giant fibers on the cross section, μm^2	3	3	3	3
Packing density of muscle fibers (number per unit of cross-sectional area), fibers/mm^2	2	3	2	3
Proportion of muscle fibers, which diameter was lower or higher by 1/3 than mean fiber diameter, %	2	2	2	3
Group 2 (8 h)				
Shape of muscle fibers	3	1	2	2
Condition of cross-striation	1	1	1	1
Mean sarcomere length, μm	3	2	2	2
Presence of destructive changes in sarcolemma	2	2	2	2
Presence of giant fibers (contraction knots), fibers/1 cm^2	2	2	1	1
Average area of giant fibers on the cross section, μm^2	3	3	3	3
Packing density of muscle fibers (number per unit of cross-sectional area), fibers/mm^2	2	3	2	2
Proportion of muscle fibers, which diameter was lower or higher by 1/3 than mean fiber diameter, %	2	3	2	2
Group 3 (10 h)				
Shape of muscle fibers	1	3	3	3
Condition of cross-striation	1	1	1	1
Mean sarcomere length, μm	2	2	3	2
Presence of destructive changes in sarcolemma	2	2	2	3
Presence of giant fibers (contraction knots), fibers/1 cm^2	1	2	2	2
Average area of giant fibers on the cross section, μm^2	2	2	3	3
Packing density of muscle fibers (number per unit of cross-sectional area), fibers/mm^2	2	3	3	3
Proportion of muscle fibers, which diameter was lower or higher by 1/3 than mean fiber diameter, %	3	3	3	3

End of Table 4

Indicator	Scoring (points) for samples from groups			
	Carcass 1	Carcass 2	Carcass 3	Carcass 4
Group 4 (16 h)				
Shape of muscle fibers	3	1	3	3
Condition of cross-striation	1	1	1	1
Mean sarcomere length, μm	2	2	2	3
Presence of destructive changes in sarcolemma	2	1	2	2
Presence of giant fibers (contraction knots), fibers/1 cm^2	1	1	1	1
Average area of giant fibers on the cross section, μm^2	2	1	2	2
Packing density of muscle fibers (number per unit of cross-sectional area), fibers/mm^2	3	2	2	2
Proportion of muscle fibers, which diameter was lower or higher by 1/3 than mean fiber diameter, %	3	2	2	2
Group 5 (18 h)				
Shape of muscle fibers	3	3	2	2
Condition of cross-striation	1	1	1	1
Mean sarcomere length, μm	2	2	2	2
Presence of destructive changes in sarcolemma	2	2	2	2
Presence of giant fibers (contraction knots), fibers/1 cm^2	2	1	2	1
Average area of giant fibers on the cross section, μm^2	2	1	3	3
Packing density of muscle fibers (number per unit of cross-sectional area), fibers/mm^2	2	2	2	2
Proportion of muscle fibers, which diameter was lower or higher by 1/3 than mean fiber diameter, %	2	2	3	3

Table 5. Final scoring of samples from the control and experimental groups by a degree of manifestation of myopathy (N = 20, n = 4)

Группы		Scoring of samples			
		Carcass 1	Carcass 2	Carcass 3	Carcass 4
№ 1 (4 h)	Total points	16	17	16	18
	Conclusion	M	P	M	P
№ 2 (8 h)	Total points	18	17	15	15
	Conclusion	P	P	M	M
№ 3 (10 h)	Total points	14	18	20	20
	Conclusion	M	P	P	P
№ 4 (16 h)	Total points	17	11	15	16
	Conclusion	P	W	M	M
№ 5 (18 h)	Total points	16	14	17	16
	Conclusion	M	M	P	M

Notes. W — Without myopathy, M — Moderately pronounced myopathy, P — Pronounced myopathy.

As scoring of samples shows (Table 5), animal groups 1, 2 and 3 were characterized by signs of moderate and pronounced myopathy. Moreover, the highest number of carcasses with pronounced myopathy was in group 3, which underwent pre-slaughter holding for 10 hours. Apparently, compared to groups 1 and 2, animals from group 3 were more strongly exhausted and subjected to stress factors. Only one carcass, which muscle tissue had signs of pronounced myopathy, was revealed in each of groups 4 and 5. With that, one carcass in group 4 did not have signs of myopathy at all. It is possible that increasing pre-slaughter holding for another two hours (from 16 to 18 hours) could negatively affect the condition of muscle tissue after slaughter.

Fasting time along with other pre-slaughter factors can influence meat quality indicators such as slaughter yield of carcasses, pH, color and water holding capacity of muscle tissue [28,29,30]. It was established that long pre-slaughter holding (36–48 hours) [31,32,33] as well as too short (less than 1 hour) [34,35] negatively affected animal welfare and pork technological characteristics. At the same time, optimal duration of pre-slaughter holding of animals without feeding continue to be a subject of scientific discussions. The recommended duration can be from 5 to 12 hours [36], 12 hours [37], from 12 to 24 hours [38].

Recommendations (16 hours) that can be made according to our results of histological investigations are in good agreement with earlier obtained data about the lowest level of cortisol in blood and urine, and therefore, the lowest stress level in pigs fasting for 12–18 hours [39] and more than 14 hours [40].

Conclusion

Microstructural investigations are a useful methodological tool for understanding a significance of an effect of one or another factor on meat quality. The study of the effect of pig's fasting time on the microstructure of chilled porcine muscle tissue, without doubt, confirmed this once more.

Analysis of the mean values of the morphometric characteristics shows that when duration of pre-slaughter holding increases, the number and area of giant fibers can decrease, which undoubtedly positively influence pork quality. The applied semi-quantitative scoring method made it possible to assess the studied samples from each carcass by a degree of manifestation of myopathic signs and confirm the positive effect of longer pre-slaughter holding.

The best results were obtained in case of total pre-slaughter holding time of 16 hours; the further increase in the duration of pre-slaughter holding did not lead to improvement of morphometric indicators of muscle tissue. With that, pre-slaughter holding of animals for 4, 8 and 10 hours led to deterioration of pork quality.

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