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EFFECT OF ADAPTOGENS ON MUSCLE TISSUE MICROSTRUCTURE OF HYBRID PIGS (*Sus scrofa domesticus* L.) DURING INTENSIVE FATTENING

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Abstract

At present, improving the quality of pork while increasing the production of pork is of generally recognized economic importance. Diets have been shown to affect the characteristics of muscle fibers. Adaptogens (vitamins and bioflavonoids) that animals need during active growth period can prevent myopathic transformation in meat. This paper for the first time reports on the improved microstructure of the *musculus longissimus dorsi* in crossbred pigs fed a complex of adaptogens (dihydroquercetin and vitamins E, C) during the fattening period. Our aim was to evaluate the effect of the complex of dihydroquercetin + vitamins E, C on the microstructure of meat muscle tissue in hybrid pigs. The experiments were carried out in 2021-2022 at Gorbatov Federal Research Center of Food Systems, Ernst Federal Research Center for Animal Husbandry and BMPK (Bryansk Province). Crossbred piglets (n = 108) (*Sus scrofa domesticus*) F2 [(Large White × Landrace) × Duroc] were randomly selected and assigned to two groups, n = 54 each, for a 58-day fattening. The control group were fed only a complex feed (SK-6, Russia), The test group was additionally fed an experimental dietary complex of adaptogens (DEC) that contains dihydroquercetin (DHQ, Ecostimul-2, JSC Ametis, Russia; 72-73 % DHQ, 32 mg/kg of feed), vitamin E (INNOVIT E60, GC MEGAMIX, Russia; 10 mg/kg feed) and vitamin C (Tiger C 35, Anhui Tiger Biotech Co. Ltd., China; 35 mg/kg feed). Young animals were weighed twice (on day 0 and day 58) by group weighing and individual weighing of 10 animals from each group. After slaughter, the paired carcass weight and the slaughter yield were assessed. To study the microstructure of muscle tissue, 45 min after slaughter, samples (3×3×3 cm) of the longest back muscle (*musculus longissimus dorsi*) were collected and fixed in 10 % neutral buffered formalin solution for 72 h at room temperature. For further study, two fragments (1.5×1.5×0.5 cm) of each sample with longitudinal and transverse orientations were washed with cold running water for 4 h, then compacted at 37 °C in gelatin (AppliChem GmbH, Germany) of ascending concentrations (12.5 and 25 %, for 8 h in each). Serial 16 μm sections were prepared using a MIKROM-HM525 cryostat (Thermo Scientific, USA), mounted on Menzel-Glaser glasses (Thermo Scientific, USA) and stained with Ehrlich's hematoxylin and 1 % eosin water-alcohol solution (BioVitrum, Russia). The histological preparations were examined and photographed using an AxioImaiger A1 light microscope (Carl Zeiss, Germany) with a connected AxioCam MRc 5 video camera (Carl Zeiss, Germany). Morphometric studies were performed using the AxioVision 4.7.1.0 image analysis program (Carl Zeiss, Germany). Muscle fiber diameter, sarcomere length, and giant fiber cross-sectional area were measured online. On cross sections, the shape of muscle fibers, their density, the state of the nuclei, the thickness and state of the connective tissue layers were investigated, and giant fibers were identified. On longitudinal sections, the state and shape of muscle fibers, the state of the sarcolemma, the presence of striation (transverse, longitudinal) and destructive changes (ruptures, cracks, fragmentation), hypercontraction nodes were identified. Despite the absence of statistically significant differences in live weight and

slaughter traits between groups during fattening, histological studies revealed significant differences in average values of muscle fiber density ($p = 0.02$) and sarcomere length ($p = 0.000007$). Almost a 2-fold decrease in the number of giant fibers in test group (11.60 vs. 21.30 pcs/cm^2) indicates a significant improvement in the tissue microstructure compared to the control animals that did not receive DEC. The less pronounced destructive changes in the sarcolemma in the test animals also indicate an increase in the animal stress resistance. Given a significant variability of morphometric parameters in both groups, we applied a scoring procedure which allows us to classify carcasses according to the severity of myopathic changes in muscle tissues based on the results of the muscle fiber microstructure study. In control, there was only one carcass that had no signs of myopathy; four carcasses showed signs of moderate myopathy and five carcasses showed signs of severe myopathy. On the contrary, in the test group, there were four carcasses without signs of myopathy and six carcasses with signs of moderate myopathy. There were no cases of severe myopathy in the study group. The groups differed statistically significantly ($p = 0.004$) in scores characterizing the severity of myopathic changes in muscle tissue. Our findings show that the dietary adaptogen complex DEC can provide the improvement of the microstructure of the muscle tissue and, therefore, has a positive effect on animal stress resistance and the degree of glycolysis in meat.

Keywords: adaptogen, dihydroquercetin, stress, young pigs, muscle tissue, giant fibers, contractile nodes, microstructure, histology

The growth of domestic pork production from 799.9 thousand tons in 2010 to 3254.92 thousand tons in 2021 was due to the industry's transition to intensive pig rearing, which was accompanied by a qualitative change in the livestock - a refusal to use traditional purebred animals. Modern intensive pig farming is characterized by the widespread distribution of hybrid fast-growing individuals, combined with a radical change in the conditions of their keeping and feeding. The main parameters of intensive production include a decrease in the age at which animals reach a live weight of 100 kg, and a high average daily weight gain [1-3]. At the same time, the quality of slaughter products, primarily pork, has changed.

Genetic progress has increased the stress on the body of fast-growing slaughter animals and led to morphological and biochemical modifications of muscle tissue, worsening the consumer and technological characteristics of meat [4)] In the carcasses of modern hybrid pigs, the content of muscle tissue exceeds 50% (wt). As the main and most nutritionally valuable part of the carcass, muscle tissue is considered the main component determining the quality of meat, which is formed during muscle metabolism before and after the death of the animal [5, 6].

Advances in the field of intensive feeding of hybrid pigs have led to the emergence of pathological features in the microstructure of muscle tissue, reducing the value of pig carcasses. The increased stress inherent in hybrid animals during transportation and lairage was considered to be the main reason for these changes [7]. The development of porcine stress syndrome leads to the fact that post-mortem redox processes in pork are characterized by increased glycolysis of muscle tissue, producing meat with signs of PSE (pale, soft and exudative)/myopathy [8]. Such meat is characterized by histopathological abnormalities in the muscles and the appearance of destructive changes (ruptures of the sarcolemma), changes in the shape of muscle fibers, the appearance of atrophied, as well as hypertrophied and giant fibers [9, 10].

In this regard, in recent years there has been renewed interest in studying the microstructural characteristics of muscle tissue of slaughter animals, including the types and properties of muscle fibers, their density and size [11-13]. It has been proven that the characteristics of muscle fibers are directly influenced by both genetic factors (breeds, lines, hybridization), as well as the growth rate of the animal and the final mass fraction of muscle tissue in the carcass [14, 15]. In turn, the characteristics of muscle tissue determine the technological and consumer quality of pork, including tenderness, juiciness and color [16]. A relationship has been shown between the rapid growth of hybrid animals and the high incidence of myopathic changes in muscle tissue, which reduce the quality of pork [17].

Muscle fibers are key components of skeletal muscle, the characteristics of which significantly influence the quality of meat [18, 19]. Histological changes in muscle tissue lead to changes in meat quality [20]. One of the signs of changes in the microstructure of muscle tissue is the appearance of giant fibers. It has been established that giant fibers are found exclusively in postmortem muscles, and most researchers believe that they arise because of overcompression of a portion of the muscle fibers [21-23]. The cause of the appearance of giant fibers is the depletion of some fibers even before slaughter and the very rapid development of rigor mortis in them, while neighboring fibers continue to remain in a relaxed state [24, 25].

Giant fibers in cross-sections of muscles are characterized by a rounded shape and a large cross-sectional area. They are most often located at the edge and, with less frequency, within the primary muscle bundles [22, 26, 27]. Giant fibers stain more intensely with eosin compared to surrounding normal fibers. In longitudinal sections, giant fibers exhibit partial to complete loss of myofibril structure as a result of hypercontraction and fiber disintegration [16, 28]. Increased giant fibers are associated with inhumane handling of animals prior to slaughter [29-31], genetic profile, and breed [31, 32]. The frequency and size of giant fibers determine the decline in pork quality [33, 34].

However, it is believed that giant fibers may not be evident in muscle tissue. For example, surrounding fibers can passively hold the affected fibers in a stretched state, preventing them from transforming into giant ones. Many authors note muscle fiber density/total number of fibers per unit cut area as an important factor determining meat quality [18, 19, 34]. This parameter is related to the diameter of the muscle fibers. On the one hand, fibers of smaller diameter are especially desirable, since they have a beneficial effect on the quality of meat and are considered an indicator of its tender structure [35], on the other hand, the presence of fibers of small diameter may be a sign of muscular dystrophy [36]. Obviously, taking into account significant deviations from the average muscle fiber diameter can be critical for objective assessment of meat quality based on histological studies [37]. Therefore, analysis of not only giant fibers, but also other morphological characteristics of muscle tissue is important [22, 25, 26].

The economic importance of improving pork quality while continuing to increase pork production is now recognized [4]. It has been shown that the choice of diet affects the characteristics of muscle fibers [38], and in preventing the appearance and development of myopathic changes in meat, an important role is played by adaptogen substances, the vitamins and bioflavonoids that animals need during the period of active growth [39-42]. Reducing the risk of receiving low-quality meat can be achieved through the use of adaptogenic drugs in vivo, which increase the stress resistance of animals and ensure optimal glycolysis in meat [34]. The search for adaptogens that act as regulators of the directional development of muscle tissue can become the main way to ensure the quality of meat while intensifying its production.

This paper is the first to report, on the example of hybrid pigs, the positive effect of a complex of adaptogens dihydroquercetin and vitamins E and C fed during the fattening period on the microstructure of *musculus longissimus dorsi*.

The purpose of the work is to assess the influence of the complex of adaptogens dihydroquercetin and vitamins E, C on the microstructural characteristics of muscle tissue of meat obtained from hybrid pigs.

Materials and methods. The experiments were carried out in 2021-2022 at Gorbatov Federal Research Center for Food Systems RAS, Ernst Federal Research Center VIZh and OOO Bryansk Meat Processing Plant (Bryansk Province).

For the experiment, 108 crossbred piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] were assigned. Live weight at the beginning of the experiment was 60-65 kg, age was 120 days. The fattening took 58 days. Experiments were performed in accordance with the principles of good laboratory practice [43-47].

All animals were kept in the same zoohygienic conditions [48] and had free access to feed and water throughout the entire study period. Feeding was carried out from group self-feeders with regard to current standards [49].

The piglets were randomly divided into two groups (control and experimental, 54 pigs in each). The control group received only SK-6 feed, balanced in nutrients and energy as recommended [49]. The experimental group was additionally fed with an experimental complex of adaptogens (DEC), containing dihydroquercetin (DHQ, Ecostimul-2, JSC Ametis, Russia; 72-73 % DHQ, 32 mg/kg of feed), vitamin E (INNOVIT E60, GC MEGAMIX, Russia; 10 mg/kg feed) and vitamin C (Tiger C 35, Anhui Tiger Biotech Co. Ltd., China; 35 mg/kg feed. The DKVES mixture was prepared in laboratory conditions and mixed with crushed wheat grain.

At a feed mill (LLC Bryansk Meat Processing Plant, Bryansk Province), a pilot batch of feed added with the DEC was produced in compliance with the approved recipes. To prepare feed with an adaptogen, the calculated amount of the complex DHQ + vitamins was weighed and mixed with the dry ingredients of the feed in a mixer, ensuring its uniform application. The addition of DEC in the amount of 0.25 kg/t of SK-6 feed did not affect the consumption of energy and nutrients. The choice of dosages was based on previously obtained own data and a generalization of current information on the problem (for more complete information, see the RF patent).

The young animals were weighed twice, before the start of the experiment and on day 58. Individual and group weighing was carried out. For comparative analysis, control slaughter and sampling, control 10 fattening pigs from each group were used (Nos. 1-10). Individual weighing was carried out during fattening and before slaughter. The control slaughter was carried out at an industrial enterprise (OOO Bryansk Meat Processing Plant).

Immediately before slaughter, the live weight (LW) of pigs after fasting was determined. After the slaughter, the weight of the carcass was determined, for treatment 1, the weight of the carcass with the head, legs and tail; for treatment 2, the weight of the carcass without the head, legs and tail). The slaughter yield was calculated as the ratio of the carcass weight to the live weight before slaughter.

For the microstructure study, 3×3×3 cm fragments of the *musculus longissimus dorsi* were taken 45 minutes after slaughter. The samples were fixed in a 10% neutral buffered formalin solution for 72 hours at 22 °C. For the study, two 1.5×1.5×0.5 cm fragments with longitudinal and transverse orientation of muscle fibers from each sample were washed with cold running water for 4 hours and compacted in 12.5% and 25% gelatin solutions (AppliChem GmbH, Germany) at 37 °C, for 8 hours in each concentration.

Serial sections with a thickness of 16 μm were prepared on a MIKROM-HM525 cryostat (Thermo Scientific, USA). Three sections were prepared from each fragment. The resulting sections were mounted on Menzel-Glaser glasses (Thermo Scientific, USA) and stained with Ehrlich hematoxylin and 1% aqueous-alcoholic eosin solution (BioVitrum, Russia) as described [50]. Histological preparations were examined and photographed (an AxioImaiger A1 light microscope with a connected AxioCam MRc 5 video camera, Carl Zeiss, Germany) [51].

For morphometric studies, the image analysis program AxioVision 4.7.1.0 (Carl Zeiss, Germany) was used. Muscle fiber diameter, sarcomere length, and

giant fiber cross-sectional area were measured. At least 100 objects were analyzed for each section. The fiber diameter was determined with an accuracy of ±1.0 μm. The length of sarcomeres was measured with an accuracy of ±0.1 μm. The number of giant fibers per 1 cm^2 section and the density of muscle fibers per 1 mm^2 were calculated.

On cross sections, the shape of muscle fibers, the density of their arrangement, the state of the nuclei, the thickness and condition of the connective tissue layers were determined, and giant fibers were identified. On longitudinal sections, the condition and shape of muscle fibers, the state of the sarcolemma, the presence of striations (transverse, longitudinal), the presence of destructive changes (ruptures, cracks, fragmentation) were determined, and hypercontraction nodes were identified.

Statistical analysis was carried out using R software (version 4.2.1). Quantitative data are presented as arithmetic means (*M*), standard errors of the mean (±SEM), standard deviations (±SD), minimum and maximum values (min/max), confidence intervals (CI) and median (*Me*). The normality of the distribution of parameters of quantitative variables was assessed using the Kolmogorov-Smirnov test. Identification of the relationship between the studied factor and morphometric parameters of muscle tissue was carried by one-way analysis of variance (ANOVA) and Dunnett's test. The differences were considered statistically significant and a relationship between the indicators was accepted at a p-level not exceeding 0.05.

Results. Animals from the control and experimental groups did not differ in either growth dynamics or slaughter indices ($p > 0.05$) (Tables 1, 2).

1. Average bodyweight of pigs (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] fed a complex of adaptogens dihydroquercetin and vitamins E, C (*M*±SEM, OOO Bryansk Meat Processing Plant, Bryansk Province, 2021-2022)

Group	Average bodyweight, kg	
	group weighing (n = 54)	individual weighing (n = 10)
	Day 0	
Control	62,41	65,7±1,4
Test	61,93	63,5±1,1
	Day 58	
Control	113,26	112,6±1,6
Test	114,33	114,1±2,1

Note. For a description of the groups, see the Materials and methods section.

2. Slaughter prameters of pigs (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] fed a complex of adaptogens dihydroquercetin and vitamins E, C (*M*±SEM, OOO Bryansk Meat Processing Plant, Bryansk Province, 2021-2022)

Group	Average carcass weight, kg		Average slaughter weight, kg	
	treatment 1 (n = 10)	treatment 2 (n = 10)	treatment 1 (n = 10)	treatment 2 (n = 10)
Control	90.7±5.1	83.0±4.7	80.5±1.3	73.6±1.3
Test	90.7±5.0	83.2±4.6	79.5±1.2	73.0±1.3

Note. For a description of the groups and treatments, see the Materials and methods section.

Nevertheless, piglets from the experimental group grew more intensively than their peers in the control group (by 6.8%, or 929.7 versus 870.7 g/day). Of the control group, 98.1% aminals remained alive vs. 100% of the test group, which confirmed the positive role of the studied nutritional factor. In both groups, carcasses did not have statistically significant differences in slaughter yield regardless of calculation mode. This indicated insignificant differences in the dynamics of animal development during the fattening period and similar mass of the resulting carcasses, both with and without by-products (head, legs, and tail).

Figure 1 shows the steps of morphometric analysis of muscle tissue in piglets.

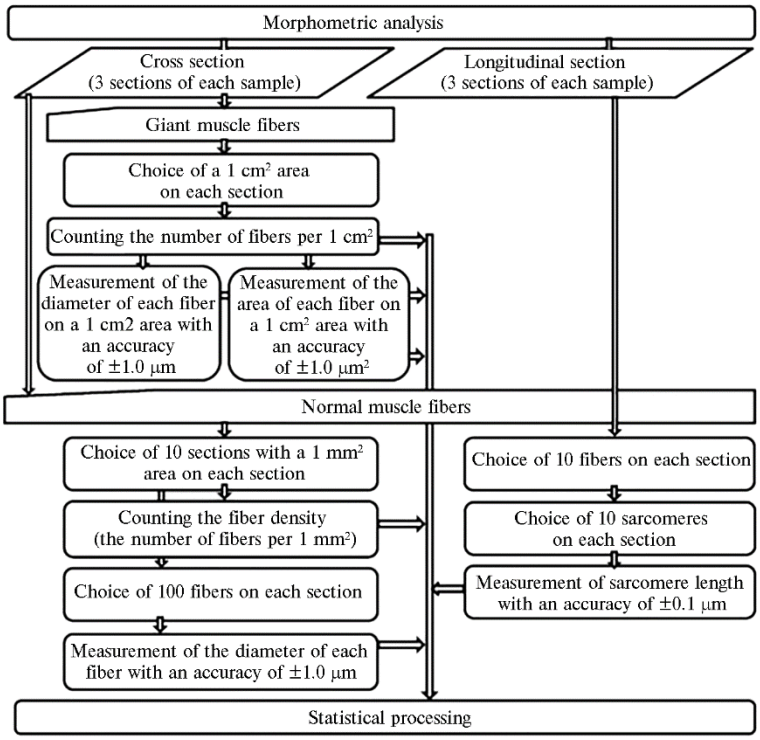
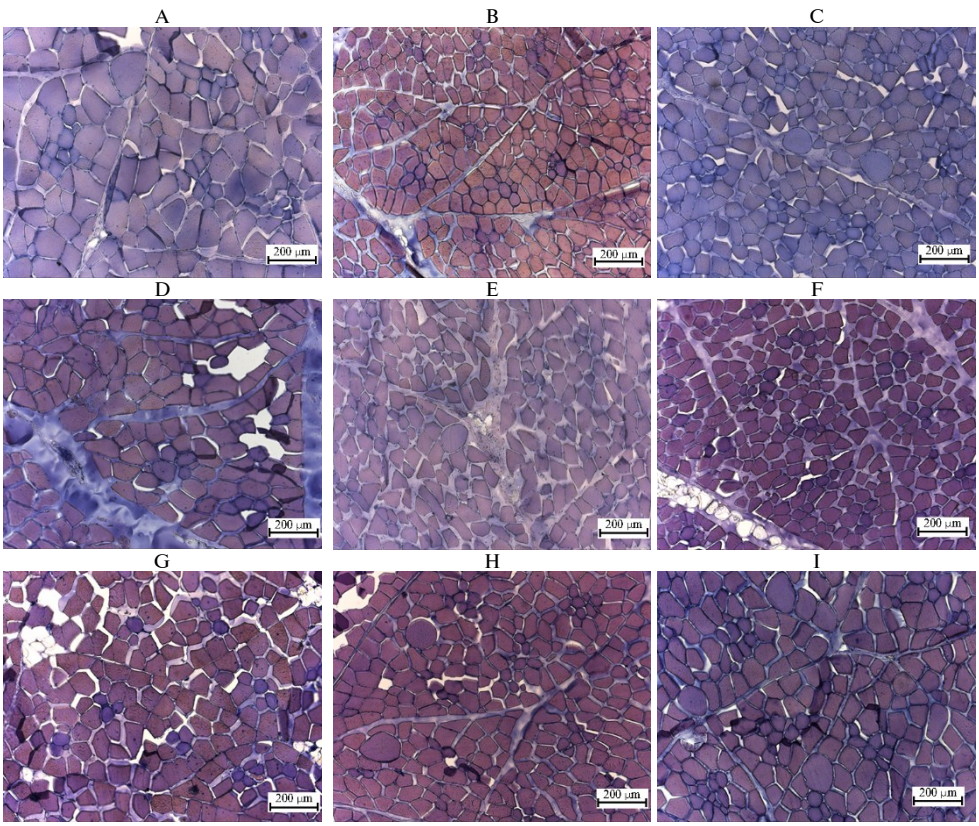


Fig. 1. The sheme of morphometric studies of muscle tissue in pigs (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] fed a complex of adaptogens dihydroquercetin and vitamins E, C (Gorbatov Federal Research Center for Food Systems RAS, 2022).



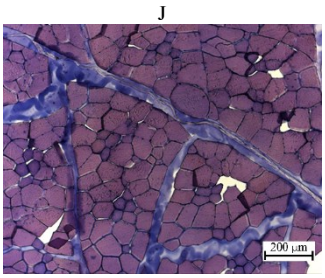


Fig. 2. Cross sections of *musculus longissimus dorsi* of crossbred piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] from the control group fed SK-6 feed: A-J — samples from carcasses Nos. 1-10 (hematoxylin and eosin staining, objective magnification ×10, eyepiece ×10, microscope AxioImaiger A1, Carl Zeiss, Germany; Gorbatov Federal Research Center for Food Systems RAS, 2022).

Despite the fact that all animals were raised, fattened and slaughtered under the same conditions, differences in the muscle tissue were observed in piglets from the control and experimental groups. Figures 2 and 3 show representative photographs of the microstructure of each of the 10 samples in the control and test groups. Figure 4 shows the structure of muscle tissue with giant fibers.

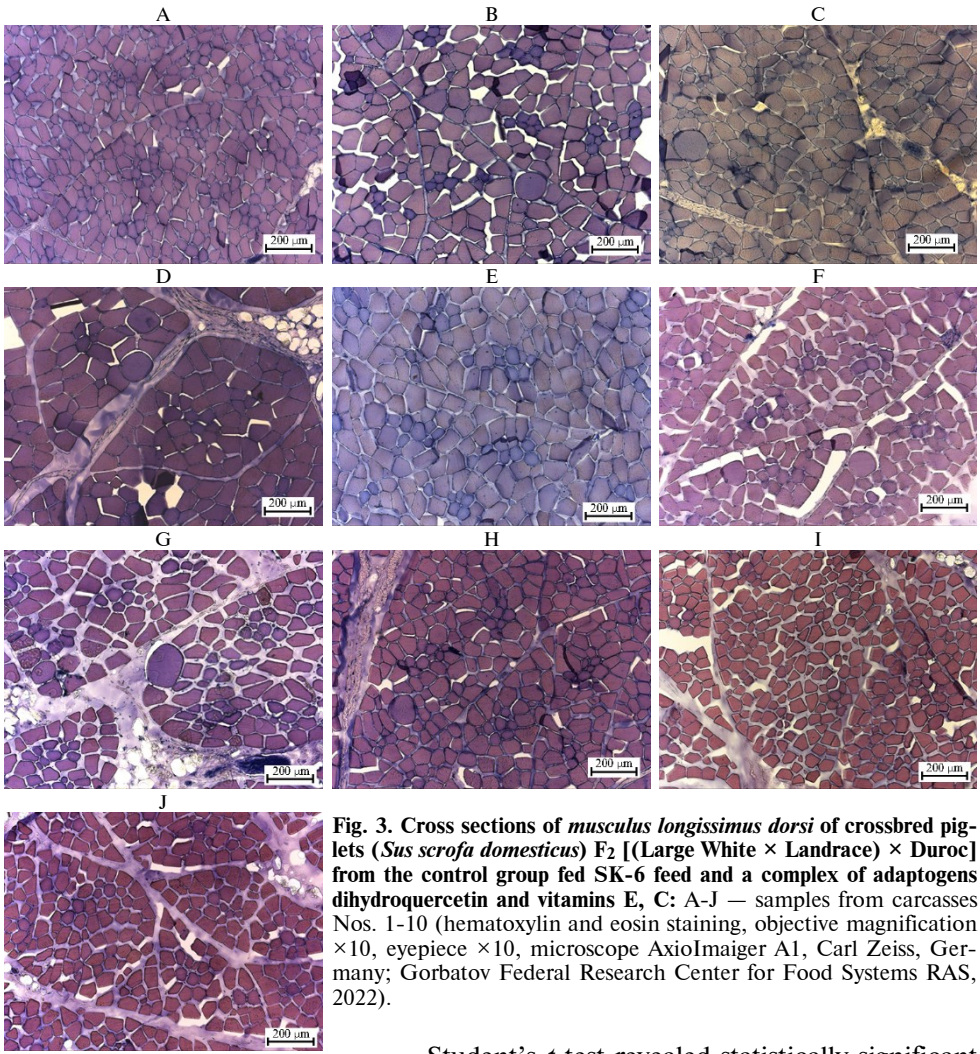


Fig. 3. Cross sections of *musculus longissimus dorsi* of crossbred piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] from the control group fed SK-6 feed and a complex of adaptogens dihydroquercetin and vitamins E, C: A-J — samples from carcasses Nos. 1-10 (hematoxylin and eosin staining, objective magnification ×10, eyepiece ×10, microscope AxioImaiger A1, Carl Zeiss, Germany; Gorbatov Federal Research Center for Food Systems RAS, 2022).

Student's *t*-test revealed statistically significant differences between the control and test groups in the average length of sarcomeres ($p = 0.000007$) and in the density of muscle fibers ($p = 0.02$) (Table 3). The medians of these prameter in the test group were greater (2.1 μm and 201/mm^2) than in the control (1.9 μm and 177/mm^2), that is, the test group had a denser arrangement of fibers and a longer sarcomere length (Fig. 5, 6).

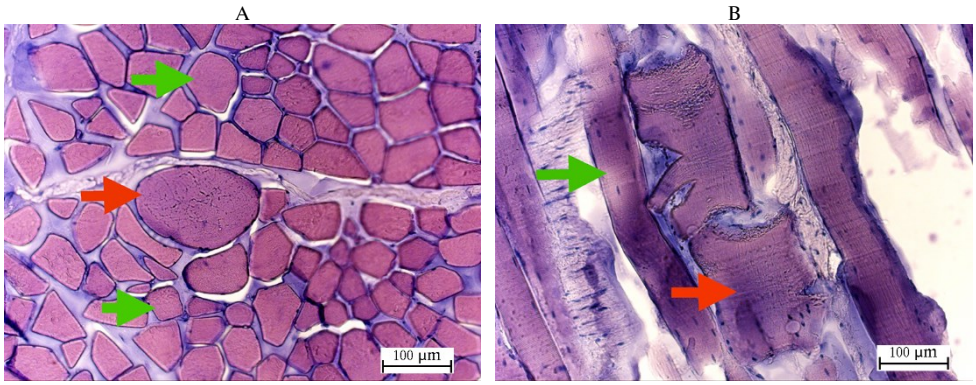


Fig. 4. Giant (red arrow) **and normal** (green arrow) **fibers in transverse** (A) **and longitudinal** (B) **sections of *musculus longissimus dorsi* of crossbred piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc].** Hematoxylin and eosin staining, objective magnification ×20, eyepiece ×10, microscope AxioImaiger A1, Carl Zeiss, Germany; Gorbatov Federal Research Center for Food Systems RAS, 2022).

3. Morphometric tissue parameters of musculus longissimus dorsi in crossbred piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Gorbatov Federal Research Center for Food Systems RAS, 2022)

Parameter		Group	
		comtrol	trst
Muscle fiber diameter, μm	k	1000	1000
	M±SD	56.8±16.7**	55.4±15.6**
	min-max	17.5-121.6	16.8-107.1
	Me	55.1	53.7
	SEM	0.5	0.5
	95 % CI	55.7-57.8	54.4-56.3
Sarcomere length, μm	k	100	100
	M±SD	1.9±0.2*	2.1±0.3*
	min-max	1.5-2.2	1.6-2.7
	Me	1.9	2.1
	SEM	0.02	0.03
	95 % CI	1.9-1.9	2.1-2.2
Muscle fiber density, number per unit cross section area (mm^2)	k	30	30
	M±SD	182±39*	206±40*
	min-max	124-265	146-286
	Me	177	201
	SEM	7	7
	95 % CI	169-198	189-218
Diameter of giant fibers, μm	k	213	116
	M±SD	123.7±14.7	121.9±15.6
	min-max	91.7-169.1	91.6-177.6
	Me	122.5	119.5
	SEM	1.0	1.5
	95 % CI	120.6-124.5	118.8-124.5
Area of giant fibers in cross section, μm^2	k	213	116
	M±SD	11999±2892	11850±3129
	min-max	6597-22460	6588-24774
	Me	11784	11206
	SEM	198	291
	95 % CI	11572-12337	11222-12358

N o t e. k — number of observations, *M* — mean, SD — standard deviation, min-max — minimum and maximum values, *Me* — median, SEM — standard error of the mean, CI — confidence interval. For a description of groups and options, see the Materials and methods section.
* and ** Differences from control are statistically significant at $p < 0,05$ and $p = 0,05$, respectively.

When examining histological sections in samples from the control and experimental groups, 213 and 116 giant fibers were identified, respectively, but no significant differences were established between the control and experimental groups in either the average diameter or the average area of giant fibers.

Average values for groups observed in biological experiments are usually not indicative of varying parameters. Even though group means are the same, traits may differ significantly in the degree and pattern of variation in specific samples within each group [53]). In our experiment, the most significant variation was

observed in the average diameter and density of muscle fibers, as well as the average area and number of giant fibers (Table 4).

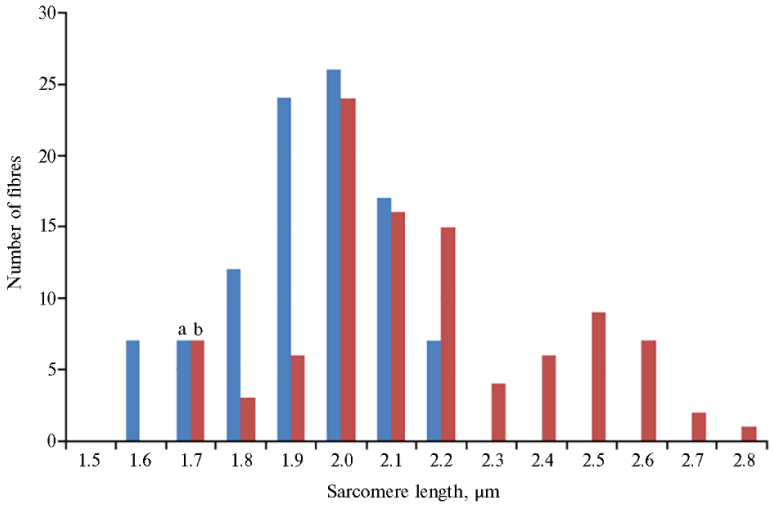


Fig. 5. Distribution of fibers by the length of sarcomeres in *musculus longissimus dorsi* of crossbred piglets (*Sus scrofa domesticus*) F2 [(Large White × Landrace) × Duroc] in control (a) **and when fed a complex of adaptogens dihydroquercetin and vitamins E, C** (b) (Gorbatov Federal Research Center for Food Systems RAS, 2022)**.**

4. Coefficient of variation (%) **of *musculus longissimus dorsi* morphometric parameters in crossbred piglets (*Sus scrofa domesticus*) F2 [(Large White × Landrace) × Duroc] fed a complex of adaptogens dihydroquercetin and vitamins E, C** (n = 10; Gorbatov Federal Research Center for Food Systems RAS, 2022)

Parameter	Group	
	control	test
Average muscle fiber diameter	29.39	31.12
Average sarcomere length	8.51	12.32
Muscle fiber density, number per unit cross section area	21.42	19.42
Average diameter of giant fibers	12.00	12.81
Average area of giant fibers on a cross section	24.11	26.40
Giant fibers density, number per unit cross section area	70.83	100.00

N o t e. For a description of groups and options, see the Materials and methods section.

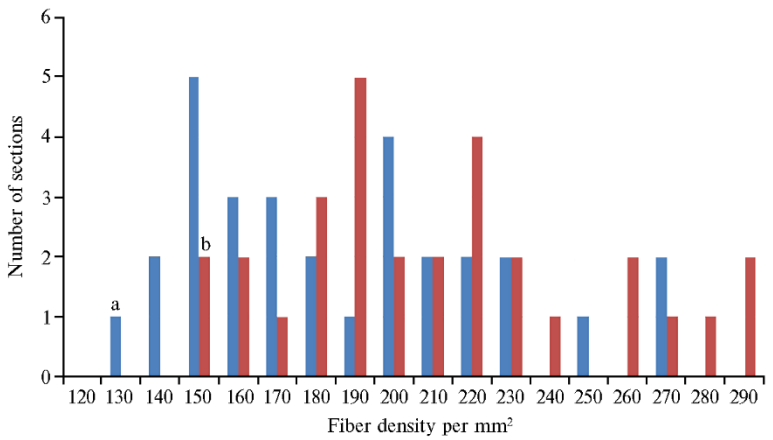


Fig. 6. Distribution of sections by the density of muscle fibers in *musculus longissimus dorsi* of crossbred piglets (*Sus scrofa domesticus*) F2 [(Large White × Landrace) × Duroc] in control (a) **and when fed a complex of adaptogens dihydroquercetin and vitamins E, C** (b) (Gorbatov Federal Research Center for Food Systems RAS, 2022)**.**

There was also a tendency towards significant differences between the control

and experimental groups in the average diameter of muscle fibers (p = 0.05), which varied respectively within the range of 55.7-57.8 and 54.4-56.3 μm. Moreover, the median in the experimental group was less than in the control group (53.7 vs. 55.1 μm), which indicated a finer-fiber structure of the samples from the experimental group. However, the nature of the distribution of the values of this indicator for the samples of the control and experimental groups was similar (Fig. 7).

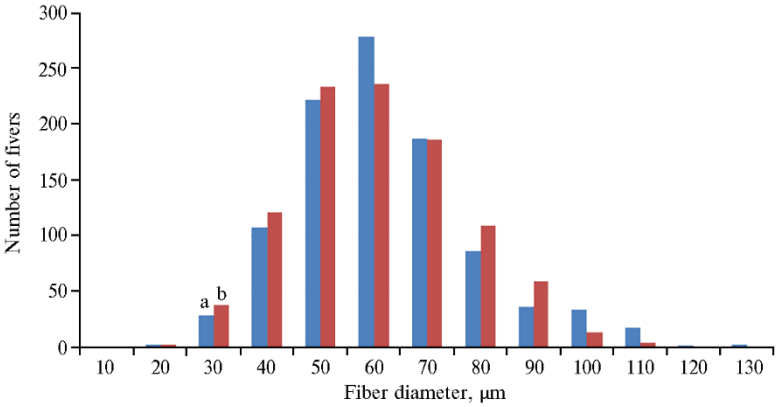
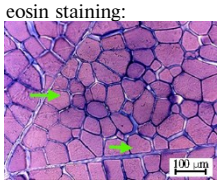
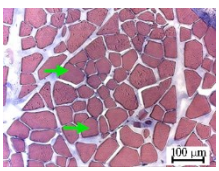
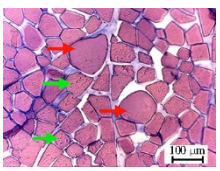


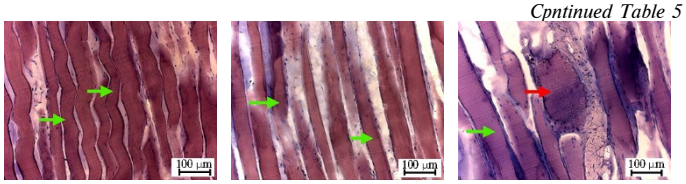
Fig. 7. Distribution of muscle fibers by diameter in *musculus longissimus dorsi* of crossbred piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] in control (a) **and when fed a complex of adaptogens dihydroquercetin and vitamins E, C** (b) (Gorbatov Federal Research Center for Food Systems RAS, 2022).

Given a significant variability of morphometric parameters, as well as the advisability of using, along with numerical data, a number of descriptive histological characteristics, we adapted and applied a semi-quantitative method based on scoring, developed jointly by the Gorbatov Federal Scientific Center for Food Systems RAS and Federal Research Center for Livestock Husbandry — Ernst VIZh [52] (Table 5).

5. Scheme for scoring the myopathy expression based on microstructural images of *musculus longissimus dorsi* of crossbred piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc]

Parameter	Description (value) of the indicator/points assigned		
	no myopathy signs	moderate myopathy	severe myopathy
Shape of muscle fibers	Slightly wavy, tightly spaced/1	Mostly straight, tightly spaced/2	Straight, lie freely in relation to each other/3
State of transverse striation	Clearly expressed/1	Small, close together, smoothed, uneven/2	Small, close together, smoothed, uneven/2
Average sarcomere length, rm	From 2.0 (inclusive) and more/1	1.6-1.9 (inclusive)/2	Less than 1.6/3
The presence of destructive changes in the sarcolemma		There are single ruptures of the sarcolemma/2	There are multiple breaks in the sarcolemma/3
Presence of giant fibers (contraction nodes), pcs/cm^2	Not detected/1	From 10 (inclusive) to 30/2	From 30 (inclusive) and more/3
Average area of giant fibers on a cross section, rm^2	Not detected and/or single detected (up to 10)/1	From 10,000 (inclusive) to 15,000/2	From 15,000 (inclusive) and more/3
Density of muscle fibers (number per unit area of cross section), pcs/mm^2	Up to 10,000/1	From 150 (inclusive) to 250/2	Up to 150/3
The proportion of muscle fibers whose diameter is less than or greater than $^1/_3$ of the average fiber diameter, %	From 250 (inclusive) and more/1	From 8 (inclusive) to 30/2	From 30 (inclusive) and more/3
Microstructure by hematoxylin and eosin staining: cross section			

Cpntinued Table 5

longitudinal section	

N o t e. Muscle tissue without signs of myopathy, with destructive changes corresponding to the normal development of autolytic processes, included samples that did not have a score of 3 points for any indicator and scored no more than 12 points inclusive. Muscle tissue with moderately expressed myopathy included samples that scored from 13 to 16 points inclusive; samples with moderate myopathy could not contain or contain individual giant fibers in the muscle tissue. Photos of the microstructure of such muscle tissue without giant fibers are shown above. Samples scoring over 16 points were classified as muscle tissue with pronounced signs of myopathy. In the photographs, green arrows indicate normal fibers, red arrows indicate giant fibers (staining with hematoxylin and eosin, objective magnification ×20, eyepiece ×10, AxioImaiger A1 microscope, Carl Zeiss, Germany).

According to the results of the scoring of the microstructure of muscle tissue (Table 6), in the control group only 1 sample was found that had no signs of myopathy, 4 samples had signs of moderate myopathy and 5 samples had signs of severe myopathy. In the test group, there were 4 samples without signs of myopathy, and 6 samples with signs of moderate myopathy. In the experimental group, not a single sample with severe myopathy was identified.

6. Individual scoring of *musculus longissimus dorsi* microstructure in crossbred piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] fed a complex of adaptogens dihydroquercetin and vitamins E, C (n = 10; Gorbatov Federal Research Center for Food Systems RAS, 2022)

Parameter	Group	Scores									
		1	2	3	4	5	6	7	8	9	10
Shape of muscle fibers	C	1	1	2	2	2	3	3	2	2	2
	T	2	2	2	1	1	3	1	1	3	3
State of transverse striation	C	1	1	1	1	1	1	1	2	2	2
	T	1	1	1	1	1	1	1	1	1	1
Average sarcomere length	C	2	2	2	2	2	1	2	2	2	3
	T	1	1	1	2	2	1	1	2	1	1
Destructive changes in the sarcolemma	C	3	1	3	1	2	1	1	3	3	3
	T	1	3	1	1	2	1	1	1	1	1
Presence of giant fibers (contraction nodes)	C	3	1	3	1	2	1	1	3	3	3
	T	1	3	2	1	1	2	2	1	1	1
Average area of giant fibers on a cross section	C	2	1	2	3	1	3	3	1	2	2
	T	1	2	2	2	2	1	2	2	1	1
Density of giant fibers (number per unit cross section area)	C	3	1	2	3	2	2	3	2	2	2
	T	1	2	2	2	2	2	3	2	1	2
Proportion of muscle fibers whose diameter is less than or greater than $^1/_3$ of the average fiber diameter	C	2	2	2	2	2	2	2	2	2	3
	T	2	2	2	2	2	2	2	2	2	1
Total points	C	17	10	17	15	14	14	16	17	18	20
	T	10	16	13	12	13	13	13	12	11	11
Conclusion about the signs of myopathy	C	S	N	S	M	M	M	M	S	S	S
	T	N	M	M	N	M	M	M	N	N	N

N o t e. C — control group, T — test group; N — no myopathy signs, M — moderate myopathy, S — severe myopathy. For a description of groups and options, see the Materials and methods section.

Statistical processing of the results of scoring the severity of signs of myopathy in muscle tissue samples (Table 7) showed that carcasses from the control and experimental groups had significant differences in the average length of sarcomeres ($p = 0.006$) and the presence of destructive changes ($p = 0.04$). According to the average number of points (see Table 7), reflecting the overall severity of myopathy, both groups differed significantly ($p = 0.004$). Nevertheless, based on the average number of points, both the control and experimental groups should be classified as samples of muscle tissue with moderately severe myopathy. However, the difference was obvious: the control group approached the state of severe myopathy (over 16 points), and the experimental group approached the state without

signs of myopathy (up to 12 points inclusive).

7. Average score (in points) **of the myopathy severity in crossbred piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] fed a complex of adaptogens dihydroquercetin and vitamins E, C** ($n = 10$, M±SEM; Gorbatov Federal Research Center for Food Systems RAS, 2022)

Parameter	Group	
	control	test
Shape of muscle fibers	2.0±0.2	1.9±0.3
State of transverse striation	1.3±0.2	1.0±0.0
Average sarcomere length	2.0±0.3*	1.3±0.2*
The presence of destructive changes in the sarcolemma	2.1±0.3*	1.3±0.2*
Presence of giant fibers (contraction nodes)	2.1±0.3	1.5±0.2
Average area of giant fibers on a cross section	2.0±0.2	1.6±0.2
Muscle fiber density (number per unit cross section area)	2.2±0.2	1.9±0.2
Proportion of muscle fibers whose diameter is less than or greater than $^1/_3$ of the average fiber diameter	2.1±0.1	1.9±0.1
Average points	15.8±0.9*	12.4±0.5*

N o t e. For a description of groups and options, see the Materials and methods section.
* Differences from control are statistically significant at $p < 0.05$.

An increasing number of myopathies have been consistently reported in animals raised intensively for meat. At the same time, there is a decline in the consumer quality of meat products [53, 54], which leads to huge economic losses for the industry. In our study, only one carcass was identified in the control group, where we did not find signs of myopathy in the muscle tissue, while half of the examined samples were classified as muscle tissue with severe myopathy. The high incidence of myopathic changes in the control group indicates the presence of a problem and confirms the concern of many researchers about the decline in the consumer characteristics of meat.

Recently, the use of adaptogens to reduce the effects of oxidative stress caused by factors associated with animal husbandry and slaughter has become increasingly popular in livestock and poultry farming [55, 56]. Polyphenols of plant origin, fat- and water-soluble vitamins are considered as adaptogens-antioxidants [57, 58]. Their effectiveness in in vivo experiments is judged mainly by blood parameters characterizing the antioxidant status of individuals receiving the adaptogen, as well as by some organoleptic qualities of meat that are important from the consumer's point of view, for example, the color of muscle tissue [59]. However, very few studies have been devoted to the study of the microstructure of muscle tissue in meat depending on the antioxidant status of animals. In this regard, our study is of interest in both theoretical and practical aspects.

To increase the antioxidant status of crossbred piglets, we used a complex of adaptogens - dihydroquercetin, vitamins E and C, which was selected based on an analysis of literature data and our own research. In the experimental group, we observed an improvement in the microstructure of muscle tissue. The muscle fibers were located more densely, the transverse striations were more pronounced, the length of the sarcomeres increased, and fewer destructive changes in the sarcolemma of the muscle fibers were observed. Muscle fibers are the key element of muscle tissue. The connection between these changes in microstructure and improved meat quality was noted in the works of other authors [60, 61]. The predominance of fibers of small and medium diameter without destructive features helps to improve the quality of meat [62]. Changes in the number and diameter of muscle fibers entail changes in meat quality [63].

The characteristics of muscle fibers depend on genetic factors [64]. Intensively growing animals obtained through two- and three-breed crossings are inferior to slow-growing purebred individuals in terms of muscle fiber density [60, 61, 63]. A positive effect of increased values of this indicator on the quality of meat

is noted [62]. In our experiment, due to the nutritional factor in animals of the same genetic origin, the F_2 [(Large White × Landrace) × Duroc] the density of muscle fibers was increased by 14% which can be considered a significant effect.

Giant fibers should be viewed as pathological consequences of past stress rather than as an additional type of normal muscle cell [23]. The presence of a large number of giant fibers in muscle tissue is also more typical for hybrid animals, suggesting the development of the PSE defect and low consumer characteristics of meat [62]. In samples of muscle tissue from piglets from the experimental group, we detected 46% fewer giant fibers.

Thus, when feeding crossbred piglets with a complex of adaptogens dihydroquercetin and vitamins E, C (DEC), significant differences were revealed between samples of the control and experimental groups in the average length of sarcomeres, the density of muscle fibers, the presence of destructive changes in the sarcolemma and the overall score of the severity of myopathic changes. The nature of the identified differences indicated an improvement in the condition of muscle tissue in animals receiving the adaptogen complex. It should be noted the effect of DEC on reducing the number of giant fibers which indicates positive changes in muscle metabolism before and after slaughter. In our experiment, factors such as genetic, breed, conditions of keeping and handling of animals were excluded. That is, the reduction in the risk of obtaining low-quality meat in the experimental group was achieved only through the use of DEC in vivo, which allows us to conclude that the adaptogen complex influences the stress resistance of animals and glycolysis in meat. The decrease in the number of giant fibers in the experimental group by almost 2 times (11.6 vs. 21.3/cm^2 in the control) indicated a significant improvement in their microstructure. The less pronounced nature of destructive changes in the sarcolemma in the experimental group also indicated an increase in the stress resistance of animals. Our studies of the muscle microstructure give reason to believe that the problem of reducing pork quality in fast-growing hybrids can be solved by coorrectly added small doses of dietary adaptogens.

REFERENCES

1. Komlatskiy G.V. *Industrializatsiya i intensifikatsiya otrasli svinovodstva na yuge Rossii. Doktorskaya dissertatsiya* [Industrial and intensive technologies of pig ferming in the south of Russia. DSc Thesis]. Cherkessk, 2014 (in Russ.).
2. Mörlein D., Link C., Werner C., Wicke M. Werner suitability of three commercially produced pig breeds in Germany for a meat quality program with emphasis on drip loss and eating quality. *Meat Science*, 2002, 77(4): 504-511 (doi: 10.1016/j.meatsci.2007.04.030).
3. *Informatsionno-tekhnicheskiy spravochnik po nailuchshim dostupnym tekhnologiyam ITS 41-2017. Intensivnoe razvedenie sviney* [Information and technical guide to the best available technologies ITS 41-2017. Intensive breeding of pigs]. Moscow, 2017 (in Russ.).
4. Petracci M., Cavani S. Muscle growth and poultry meat quality issues. *Nutrients*, 2012, 4(1): 1-12 (doi: 10.3390/nu4010001).
5. Szulc K., Wojtysiak D., Migdał Ł., Migdał W. The muscle fibre characteristics and the meat quality of *m. longissimus thoracis* from polish native złotnicka spotted pigs and the crossbreed fatteners from the crossing of Duroc and Polish Large White boars. *Appl. Sci.*, 2022, 12(6): 3051 (doi: 10.3390/app12063051).
6. Listrat A., Lebret B., Louveau I., Astruc T., Bonnet M., Lefaucheur L., Bugeon J. Comment la structure et la composition du muscle déterminent la qualité des viands ou chairs. *INRAE Productions Animales*, 2015, 28(2): 125-136 (doi: 10.20870/productions-animales.2015.28.2.3020).
7. Vermeulen L., Van de Perre V., Permentier L., De Bie S., Verbeke G., Geers R. Pre-slaughter sound levels and pre-slaughter handling from loading at the farm till slaughter influence pork quality. *Meat Science*, 2016, 116: 86-90 (doi: 10.1016/j.meatsci.2016.02.007).
8. Scheffler T.L., Gerrard D.E. Mechanisms controlling pork quality development: The biochemistry controlling postmortem energy metabolism. *Meat Science*, 2007, 77(1): 7-16 (doi: 10.1016/j.meatsci.2007.04.024).
9. Fiedler I., Ender K., Wicke M., Maak S., von Lengerken G., Meyer W. Structural and functional

characteristics of muscle fibres in pigs with different malignant hyperthermia susceptibility (MHS) and different meat quality. *Meat Science*, 1999, 53(1): 9-15 (doi: 10.1016/s0309-1740(99)00030-3).
10. Fiedler I., Dietl G., Rehfeldt C., Wegner J., Ender K. Muscle fibre traits as additional selection criteria for muscle growth and meat quality in pigs — results of a simulated selection. *Journal of Animal Breeding and Genetics*, 2004, 121(5): 331-344 (doi: 10.1111/j.1439-0388.2004.00466.x).
11. Brooke M.H., Kaiser K.K. Muscle fiber types: How manyand what kind? *Arch. Neurol.*, 1970, 23: 369-379 (doi: 10.1001/archneur.1970.00480280083010).
12. Greaser M.L., Okochi H., Sosnicki A.A. Role of fiber types in meat quality. *Proc. 47th ICoMST*. Cracow, Poland, 2001: 34-37.
13. Karlsson A.H., Klont R.E., Fernadez X. Skeletal muscle fibres as factors for pork quality. *Livestock Production Science*, 1999, 60(2-3): 255-269 (doi: 10.1016/S0301-6226(99)00098-6).
14. Cameron N.D., Oksbjerg N., Henckel P. Muscle fibre characteristics of pigs selected for components of efficient lean growth rate. *Proceedings of the British Society of Animal Science*, 1997: 32 (doi: 10.1017/S1752756200594733).
15. Cameron N.D., Oksbjerg N., Henckel P., Nute G., Brown S., Wood J.D. Relationships between muscle fibre traits with meat and eating quality in pigs. *Proceedings of the British Society of Animal Science*, 1998: 123 (doi: 10.1017/S1752756200597750).
16. Wegner J., Ender K. Microstructural bases for the growth of muscle and fatty tissue and relation to meat deposit and meat quality. *Fleischwirtschaft*, 1990, 70: 337-340.
17. Semenova A.A., Kuznetsova T.G., Nasonova V.V., Nekrasov R.V., Bogolyubova N.V. Myopathy as a destabilizing factor of meat quality formation. *Theory and Practice of Meat Processing*, 2019, 4(3): 24-31 (doi: 10.21323/2414-438X-2019-4-3-24-31).
18. Ryu Y.C., Choi Y.M., Lee S.H., Shin H.G., Choe J.H., Kim J.M., Hong K.C., Kim B.C. Comparing the histochemical characteristics and meat quality traits of different pig breeds. *Meat Science*, 2008, 80(2): 363-369 (doi: 10.1016/j.meatsci.2007.12.020).
19. Wojtysiak D., Połtowicz K. Carcass quality, physico-chemical parameters, muscle fibre traits and myosin heavy chain composition of *m. longissimus lumborum* from Pulawska and Polish Large White pigs. *Meat Science*, 2014, 97(4): 395-403 (doi: 10.1016/j.meatsci.2014.03.006).
20. Damez J.-L., Clerjon S. Meat quality assessment using biophysical methods related to meat structure. *Meat Science*, 2008, 80(1): 132-149 (doi: 10.1016/j.meatsci.2008.05.039).
21. Dutson T.R., Merkel R.A., Pearson A.M., Gann G.L. Structural characteristics of porcine skeletal-muscle giant myofibers as observed by light and electron microscopy. *Journal of Animal Science*, 1978, 46(5): 1212-1220 (doi: 10.2527/jas1978.4651212x).
22. Sink J.D., Mann O.M., Turgut H. Characterization of the giant myofiber in bovine skeletal muscle. *Experimental Cell Biology*, 1986, 54(1): 1-7 (doi: 10.1159/000163337).
23. Sosnicki A. Histopathological observation of stress myopathy in M. longissimus in the pig and relationships with meat quality. fattening and slaughter traits. *Journal of Animal Science*, 1987, 65(2): 584-596 (doi: 10.2527/jas1987.652584x).
24. Schubert-Schoppmeyer A., Fiedler I., Nurnberg G., Jonas L., Ender K., Maak S., Rehfeldt C. Simulation of giant fibre development in biopsy samples from pig longissimus muscle. *Meat Science*, 2008, 80(4), 1297-1303 (doi: 10.1016/j.meatsci.2008.06.008).
25. Dalle Zotte A., Remignon H., Ouhayoun J. Effect of some biological and zootechnical factors on appearance of giant fibres in the rabbit. Consequences on muscle fibre type, morphology and meat quality. *World Rabbit Science*, 2001, 9: 1-7 (doi: 10.4995/wrs.2001.439).
26. Remignon H., Zanusso J., Albert G., Babilé R. Occurrence of giant myofibres according to muscle type, pre- or post-rigor state and genetic background in turkeys. *Meat Science*, 2002, 56(4): 337-343 (doi: 10.1016/s0309-1740(00)00059-0).
27. Dalle Zotte A., Tasoniero G., Puolanne E., Remignon H., Cecchinato M., Catelli E., Cullere M. Effect of "Wooden Breast" appearance on poultry meat quality, histological traits, and lesions characterization. *Czech J. Anim. Sci.*, 2017, 62: 51-57 (doi: 10.17221/54/2016-CJAS).
28. Wojtysiak D., Poltowicz K., Migdal W. Effect of breed and age on histopathological changes in pig *m. semimembranosus*. *Annals of Animal Science*, 2012, 12(3): 311-321 (doi: 10.2478/v10220-012-0026-2).
29. Bee G., Biolley C., Guex G., Herzog W., Lonergan S.M., Huff-Lonergan E. Effects of available dietary carbohydrate and preslaughter treatment on glycolytic potential, protein degradation, and quality traits of pig muscles. *Journal of Animal Science*, 2006, 84(1): 191-203 (doi: 10.2527/2006.841191x).
30. Küchenmeister U., Nürnberg K., Fiedler I., Kuhn G., Nürnberg G., Ender K. Cell injury and meat quality of Pig in the time period post mortem from two genotypes susceptible or resistant to malignant hyperthermia. *European Food Research and Technology*, 1999, 209: 97-103 (doi: 10.1007/s002170050465).
31. Wojtysiak D., Gyrska M., Wojciechowska J. Muscle fibre characteristics and physico-chemical parameters of *m. semimembranosus* from pulawska, Polish Large White and Pietrain pigs. *Folia Biologica (Kraków)*, 2016, 64(3): 197-204 (doi: 10.3409/fb64_3.197).
32. Rey-Salgueiro, L., Martinez-Carballo E., Fajardo P., Chapela M.J., Espiceira M., Simal-Gandara J. Meat quality in relation to swine well-being after transport and during lairage at the slaughterhouse. *Meat Science*, 2018, 142: 38-43 (doi: 10.1016/j.meatsci.2018.04.005).
33. Sobczak M., Lachowicz K., Żochowska-Kujawska J. The influence of giant fibres on utility for

production of massaged products of porcine muscle *longissimus dorsi*. *Meat Science*, 2010, 84: 638-644 (doi: 10.1016/j.meatsci.2009.10.024).
34. Ostrenko K.S., Lemeshevsky V.O., Ovcharova A.N., Galochkina V.P., Sofronova O.V. Effect of adaptogens on the quality of pig meat. *Ukrainian Journal of Ecology*, 2020, 10(1): 344-348 (doi: 10.15421/2020_54).
35. Kasprzyk A., Bogucka J. Meat quality of Pulawska breed pigs and image of longissimus lumborum muscle microstructure compared to commercial DanBred and Naima hybrids. *Arch. Anim. Breed.*, 2020, 63(2): 293-301 (doi: 10.5194/aab-63-293-2020).
36. Golovakha V.I., Piddubnyak O.V., Shulyak V.V., Petrenko A.S., Patsenko D.A., Patsenko E.V. *Uchenye zapiski uchrezhdeniya obrazovaniya Vitebskaya ordena Znak pocheta gosudarstvennaya akademiya veterinarnoy meditsiny*, 2015, 51(1-1): 21-25 (in Russ.).
37. Bogolyubova N.V., Kuznetsova T.G., Nasonova V.V. i dr. *Sposob otsenki kachestva svininy. A.s. 2770804 (RF) S1 MPK G01N 33/12. Federal'noe issledovatel'skiy tsentr zhivotnovodstva -VIZh imeni akademika L.K. Ernsta" (RF), № 2021123212. Zayavl. 04.08.2021. Opubl. 21.04.2022. Byul. № 12* [Method for assessing the quality of pork. A.s. 2770804 (RF) C1 IPC G01N 33/12. Ernst Federal Research Center for Animal Husbandry (Russian Federation), №. 2021123212. Appl. 08/04/2021. Publ. 04/21/2022. Bull. № 12] (in Russ.).
38. Joo S.T., Kim G.D., Hwang Y.H., Ryu Y.C. Control of fresh meat quality through manipulation of muscle fiber characteristics. *Meat Science*, 2013, 95(4): 828-836 (doi: 10.1016/j.meatsci.2013.04.044).
39. Rice D.A. Kennedy S. Vitamin E, selenium, and polyunsaturated fatty acid concentrations and glutathione peroxidase activity in tissues from pigs with dietetic microangiopathy (mulberry heart disease). *American Journal of Veterinary Research*, 1989, 50(12): 2101-2104.
40. Li W.J., Zhao G.P., Chen J.L., Zheng M.Q., Wen J. Influence of dietary vitamin E supplementation on meat quality traits and gene expression related to lipid metabolism in the Beijing-you chicken. *British Poultry Science*, 2009, 50(2): 188-98.1 (doi: 10.1080/00071660902755409).
41. Semenova A.A., Nasonova V.V., Kuznetsova T.G., Tunieva E.K., Bogolyubova N.V., Nekrasov R.V. Psiii-17 program chair poster pick: a study on the effect of dihydroquercetin added into a diet of growing pigs on meat quality. *Journal of Animal Science*, 2020, 98(S4): 364 (doi: 10.1093/jas/skaa278.639).
42. Semenova A.A., Kuznetsova T.G., Nasonova V.V., Nekrasov R.V., Bogolyubova N.V., Tsis E.Yu. Use of antioxidants as adaptogens fed to pigs (*Sus scrofa domesticus* Erxleben, 1777) (meta-analysis). *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2020, 55(6): 1107-1125 (doi: 10.15389/agrobiology.2020.6.1107eng).
43. *Direktiva 2010/63/EC Evropeyskogo parlamenta i Soveta Evropeyskogo soyuza po okhrane zhivotnykh, ispol'zuemykh v nauchnykh tselyakh*. St. Petersburg, 2012. Available: https://ruslasa.ru/wp-content/uploads/2017/06/Directive_201063_rus.pdf. No date.
44. *European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS № 123) (Strasburg, 18.03.1986)*. Available: https://norecopa.no/legislation/council-of-europe-convention-ets-123. Accessed: 20.07.2022.
45. *GOST 33215-2014. Rukovodstvo po soderzhaniyu i ukhodu za laboratornymi zhivotnymi. Pravila oborudovaniya pomeshcheniy i organizatsii protsedur* [GOST 33215-2014. Guidelines for the maintenance and care of laboratory animals. Rules for equipping premises and organizing procedures]. Moscow, 2016 (in Russ.).
46. *GOST 33647-2015. Printsipy nadlezhashchey laboratornoy praktiki (GLP). Terminy i opredeleniya* [GOST 33647-2015. Principles of good laboratory practice (GLP). Terms and definitions]. Moscow, 2019 (in Russ.).
47. *GOST 33044-2014. Printsipy nadlezhashchey laboratornoy praktiki* [GOST 33044-2014. Principles of good laboratory practice]. Moscow, 2019 (in Russ.).
48. *Metodologiya nauchnykh issledovaniy v zhivotnovodstve* /Pod redaktsiey V.S. Antonovoy, G.M. Topurii, V.I. Kosilova [Methodology of research in animal husbandry. V.S. Antonova, G.M. Topurii, V.I. Kosilov (eds.)]. Orenburg, 2011 (in Russ.).
49. Nekrasov R.V., Golovin A.V., Makhaev E.A., Anikin A.S., Pervov N.G., Strekozov N.I., Mysik A.T., Duborezov V.M., Chabaev M.G., Fomichev Yu.P., Gusev I.V. *Normy potrebnostey molochnogo skota i sviney v pitatel'nykh veshchestvakh* /Pod redaktsiey R.V. Nekrasova, A.V. Golovina, E.A. Makhaeva [Nutrient requirements for dairy cattle and pigs. R.V. Nekrasov, A.V. Golovin, E.A. Makhaev (eds.)]. Moscow, 2018 (in Russ.).
50. Romeis B. *Mikroskopische technic*. P. Böck (ed.). Urban und Schwarzenberg, Münche—Wien—Baltimore, 1989 (doi: 10.1002/jobm.3620300221).
51. *Matematicheskie metody v biologii i ekologii: vvedenie v elementarnuyu biometriyu* /Pod redaktsiey S.I. Sideleva [Mathematical methods in biology and ecology: an introduction to elementary biometrics. S.I. Sidelev (ed.)]. Yarostavl', 2012 (in Russ.).
52. Semenova A.A., Nasonova V.V., Kuznetsova T.G., Nekrasov R.V. *Provedenie gistologicheskikh issledovaniy po vyyavleniyu miopatii. MR 001-00496254/00419779-2021* [Histological studies to identify myopathy. MP 001-00496254/00419779-2021]. Moscow, 2021 (in Russ.).
53. Kuttappan V.A., Hargis B.M., Owens C.M. White striping and woody breast myopathies in the modern poultry industry: a review. *Poultry Science*, 2016, 95(11): 2724-2733 (doi: 10.3382/ps/pew216).

54. Wojtysiak D. Pathological changes in the microstructure of *longissimus lumborum* muscle from five breeds of pigs. *Folia Biologica*, 2012, 60(1-2): 55-60 (doi: 10.3409/FB60_1-2.55-60).
55. Pirgozliev V.R., Mansbridge S.C., Westbrook C.A., Woods S.L., Rose S.P., Whiting I.M., Yovchev D.G., Atanasov A.G., Kljak K., Staykova G.P., Ivanova S.G., Karagecili M.R., Karadas F., Stringhini J.H. Feeding dihydroquercetin and vitamin E to broiler chickens reared at standard and high ambient temperatures. *Archives of Animal Nutrition*, 2020, 74(6): 496-451 (doi: 10.1080/1745039x.2020.1820807).
56. Zou Y., Wei H.K., Xiang Q.-H., Wang J., Zhou Y.-F., Peng J. Protective effect of quercetin on pig intestinal integrity after transport stress is associated with regulation oxidative status and inflammation. The *Journal of Veterinary Medical Science*, 2016, 78(6): 1487-1494 (doi: 10.1292/jvms.16-0090).
57. Williamson G., Kay C.D., Crozier A. The bioavailability, transport, and bioactivity of dietary flavonoids: a review from a historical perspective. *Comprehensive Reviews in Food Science and Food Safety*, 2018, 17(5): 1054-1112 (doi: 10.1111/1541-4337.12351).
58. Peeters E. Neyt A., Beckers F., De Smet S., Aubert A.E., Geers R. Influence of supplemental magnesium, tryptophan, vitamin C, and vitamin E on stress responses of pigs to vibration. *Journal of Animal Science*, 2005, 83(7): 1568-1580 (doi: 10.2527/2005.8371568x).
59. Gao K., Luo Z., Han S., Li Z., Choe H.M., Paek H., Quan B., Kang J., Yin X. Analysis of meat color, meat tenderness and fatty acid composition of meat in second filial hybrid offspring of MSTN mutant pigs. *Meat Science*, 2022, 193: 108929 (doi: 10.1016/j.meatsci.2022.108929).
60. Lebedová N., Okrouhlá M., Zadinová K., Čítek J., Stupka R. Muscle fibre composition and meat quality in pigs with different nutrition level. *IOP Conference Series: Materials Science and Engineering*, 2018, 420: 012078 (doi: 10.1088/1757-899X/420/1/012078).
61. Lebedová N., Stupka R., Čítek J., Okrouhlá M., Zadinová K. Effect of feed restriction on muscle fibre characteristics and meat quality traits in pigs. *Agronomy Research*, 2019, 17(1): 176-185 (doi: 10.15159/ar.19.004).
62. Kim G.-D., Jeong J.-Y., Jung E.-Y., Yang H.-S., Lim H.-T., Joo S.-T. The influence of fiber size distribution of type IIB on carcass traits and meat quality in pigs. *Meat Science*, 2013, 94(2): 267-273 (doi: 10.1016/j.meatsci.2013.02.001).
63. Weng K., Huo W., Li Y., Zhang Y., Zhang Y., Chen G., Xu Q. Fiber characteristics and meat quality of different muscular tissues from slow- and fast-growing broilers. *Poultry Science*, 2021, 101(1): 101537 (doi: 10.1016/j.psj.2021.101537).
64. Shen L., Luo J., Lei H., Jiang Y.Z., Bai L., Li M.Z., Tang G., Li X.W., Zhang S.H., Zhu L. Effects of muscle fiber type on glycolytic potential and meat quality traits in different Tibetan pig muscles and their association with glycolysis-related gene expression. *Genetics and Molecular Research*, 2015, 14(4): 14366-14378 (doi: 10.4238/2015.November.13.22).