

EFFECT OF MODELLED STRESS AND ADAPTOGENS ON MICROSTRUCTURAL CHARACTERISTICS OF PORK FROM FAST-GROWING HYBRID ANIMALS

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ABSTRACT

This research aimed to study the effect of modelled technological stress and the introduction of selenium and dihydroquercetin (DHQ) into pig diets on the microstructure of *M. longissimus dorsi* muscle tissue. The *in vivo* experiment was carried out on 36 hybrid young barrows (Large White x Landrace) x Duroc) with an initial live weight of 34 – 36 kg until they reached a weight of not less than 110 kg. The animals were divided into four groups: 1 (C-) – pigs did not receive adaptogens and were not subjected to modelled technological stress; 2 (C+) – pigs did not receive adaptogens but were subjected to stress via relocation of animals; 3 (C+Se) – pigs were subjected to stress and received 0.2 mg Se.kg⁻¹ feed as selenium proteinate in addition to their diet; 4 (C+DHQ) – pigs were subjected to stress and received 32 mg DHQ.kg⁻¹ feed in addition to their diet. The best results regarding the muscle tissue condition were recorded in the muscle *L. dorsi* samples were taken from the carcasses of group 4 (C+DHQ). Analysis of variance using the Fisher–Snedecor test confirmed that addition of adaptogens led to an improvement of the pH₂₄ value (at $p = 0.05$, $f_{observed} = 5.90 > f_{critical} = 4.17$) and moisture-holding capacity (at $p = 0.05$, $f_{observed} = 3.04 > f_{critical} = 2.92$). The effect of long-term addition of DHQ to pig diets (78 days) on the condition of muscle tissue was studied for the first time, which allowed us to conclude its role in the prevention of myopathic changes in the muscle fibre structure.

Keywords: pork; muscle tissue; myopathy; adaptogen; stress

INTRODUCTION

It is known that the development of degenerative (myopathic) changes in the muscle tissue structure which affect pork quality is a consequence of lifetime stress on the animal and/or inadequate nutrition (Semenova et al., 2019). All types of mammals are exposed myopathic changes (Herraez et al., 2013). Pig breeding and poultry farming have the most economic losses due to myopathic. On poultry farms, up to 70% of the poultry stock is affected by myopathy, which manifests itself on the breast muscles (Kijowski and Kupinska, 2012). Hybrid fast-growing pigs are more sensitive to stress. In these animals, nodes, and bands of super contraction (or hyper contraction), the appearance of which leads to deterioration of pork technological and consumer quality, are seen in muscles after slaughter.

Therefore, the aim of this research was to study the effect of modelled technological stress and the introduction of adaptogens (selenium and dihydroquercetin (DHQ)) into pig diets on the muscle tissue microstructure of *M. longissimus dorsi*.

Scientific hypothesis

The muscles of fast-growing hybrid pigs are characterized by signs of myopathy, which develop in conditions of limited animal movement and under the influence of stress. Destructive changes in muscle tissue leading to a decrease in meat quality. Use adaptogens into feed can reduce the severity of signs of myopathy.

MATERIAL AND METHODOLOGY

The experiment was carried out *in vivo* on 36 hybrid young barrows (F-2 hybrids: (Large White x Landrace) x Duroc)) with an initial live weight of 34 to 36 kg. The animals were kept in the physiological yard of the L.K. Ernst Federal Science Center for Animal Husbandry (VIZh) from May 21 to September 28, 2019. Animals in all groups were kept under the same conditions (temperature, humidity and light regimes, the gas composition of the air inside the building), which corresponded to the zoohygienic norms in all groups. Animals were fed according to the norms of VIZh (Nekrasov, Golovin and Makhayev, 2018). The animals were selected according to the pair-analogue principle and divided into four groups with nine animals in each group:

- control group 1 (C-) – pigs did not receive adaptogens and were not subjected to modelled technological stress;
- control group 2 (C+) – pigs did not receive adaptogens but were subjected to stress after the nursery period until slaughter;
- experimental group 3 (C+Se) – pigs were subjected to similar stress and received selenium proteinate in an amount of 20 mg.kg⁻¹ feed (or 0.2 mg Se.kg⁻¹ feed) additionally to their diet throughout the experiment until slaughter;
- experimental group 4 (C+DHQ) – pigs were subjected to similar stress and received the dihydroquercetin preparation in an amount of 40 mg.kg⁻¹ feed (or 32 mg DHQ.kg⁻¹ feed) additionally to their diet throughout the experiment until slaughter.

To create a stress factor in groups C+, C+Se and C+DHQ, the animals were kept in three pens with three pigs in each and were subjected to relocation after 14 days, which resulted in new “neighbors” in a pen (Table 1). In control group 1, animals were also kept in pens with three pigs per pen, but they were not relocated. Each pen corresponded to the norms of pig keeping (concrete floor (1.5 × 2.0 m) with a rubber cover (1.5 × 1.0 m) and was equipped with a nipple drinker and a group feeder with two dividers.

The period of pig fattening consisted of two stages with a diet corresponding to the physiological requirements of animals. The duration of the stages were 42 and 36 days, respectively. Animals were slaughtered using the so-called “peasant method” after they reached a live weight of not less than 110 kg under the conditions of the physiological yard. The pigs were immobilized with a powder-charged handheld captive bolt stunner with a shortened bolt. Samples of muscle tissue were taken from the *M. longissimus dorsi* of chilled carcasses at the level of the last thoracic vertebra and the 6th and 7th lumbar vertebrae.

The following methods were used in the study:

- live weight and weight of hot carcasses were measured by weighing each animal (hot carcass) on REUS-A-PK brand and an error of weight measurement (scale interval) of 0.1 kg; weighing scales with a possible load range of 0 to 300; weighing scales with a possible load range of 0 to 300 kg;
- backfat thickness was measured using a ruler according to **GOST 427-75 (1977)** by measuring backfat thickness over spinous processes between the 6th and 7th thoracic vertebrae without counting hide thickness;
- carcass category was determined depending on carcass weight and backfat thickness according to **GOST 31476 (2012)**;

- pH was measured by the potentiometric method using a pH meter “Teslo 205” with a measurement error of ±0.02. Chilled carcasses were sorted by the mean pH value obtained after statistical processing of the results of not less than five parallel measurements in *M. longissimus dorsi*;
- moisture-holding capacity (MHC) was determined using the press method by Grau and Hamm with the modifications of Volovinskaya (**Zhuravskaya, Alekhina and Otryashnikova, 1985**);
- microstructural investigations were carried out according to **GOST 19496-93 (1995)**; muscle tissue types were determined using Sudan black staining.

The research was approved by the bioethical commission of the V.M. GorbatoV Federal Research Center for Food Systems of the Russian Academy of Sciences (protocol #03/2019, dated May 31, 2019).

Statistical analysis

Statistical analysis was carried out by least-squares mean comparisons using the PDIFF option of the general linear model procedure (SAS, 2002; SAS Inst. Inc., Cary, NC, USA). Each pig was considered as an experimental unit in measuring growth performance, while an individual sample of pork muscle tissue was used as an experimental unit for analyzing functional technological indices and ratio of different muscle fibre types in *M. longissimus dorsi*. The differences in MHC between groups were confirmed statistically using the Fisher–Snedecor test (F-distribution at $p = 0.05$, degrees of freedom: 3 and 31; $f_{observed} = 3.04$, $f_{critical} = 2.92 \Rightarrow f_{observed} > f_{critical}$). Statistical differences were considered highly significant at $p < 0.01$, significant at $p < 0.05$ and as a tendency between $p \geq 0.05$ and $p \leq 0.10$). The obtained experimental data were processed biometrically with STATISTICA (vers. 10, StatSoft, Inc., 2011).

RESULTS AND DISCUSSION

During fattening, all four pig groups showed good results expressed as intensive growth; therefore, animal groups did not display statistically significant differences in live weight at any fattening stage nor in hot carcass weight (Table 2).

The fact that live animal weight and carcass weight in all four groups, including the animals that received adaptogens, did not show significant differences and all carcasses were assigned to the 2nd category is in good agreement with the results of foreign studies from different years (**Mahan, Cline and Richert, 1999; Ivanova et al., 2019**).

Table 1 A scheme of animal relocation to create a stress factor by the example of one group (control group).

Date	Subgroup 2.1.			Subgroup 2.2.			Subgroup 2.3.		
02.07.19	1	2	3	4	5	6	7	8	9
16.07.19	1	4	7	2	5	8	3	6	9
30.07.19	3	5	7	1	6	9	2	4	8
13.08.19	2	6	9	3	4	7	1	5	8
27.08.19	1	4	9	2	5	8	3	6	7

Note: (M ±m, n = 9).

Table 2 Live weight of the experimental pigs, weight of hot carcasses and backfat thickness in the control and experimental groups.

Item	Mean value for groups			
	Control group 1, C-	Control group 2, C+	Experimental group 3, C+Se	Experimental group 4, C+DHQ
Live weight at the beginning of fattening, kg	34.71 ±1.24	34.64 ±1.68	34.89 ±0.96	34.51 ±1.11
Live weight at the end of the 1 st period, kg	74.33 ±1.85	74.90 ±2.45	73.59 ±0.98	74.43 ±1.85
Live weight at the end of fattening, kg	116.19 ±1.83	115.42 ±1.51	113.03 ±0.56	115.77 ±1.39
Live weight after fasting, kg	109.86 ±1.54	109.76 ±1.36	106.90 ±0.90	109.61 ±1.34
Hot carcass weight, kg	80.04 ±1.48	78.31 ±2.68	78.71 ±0.84	79.91 ±1.09
Backfat thickness, mm	20.44 ±1.45	20.78 ±0.20	20.44 ±1.40	21.33 ±2.05

Note: * – without head, legs, tail, internal organs and internal fat, ** – between the 6th and 7th thoracic vertebrae of the left half-carcass without hide thickness.

Uniformity of the studied animals by weight was important for the following investigation of the effect of stress and adaptogens on muscle tissue microstructure. For example, when studying myopathy in fast-growing chickens and turkeys, it was established that poultry weight affected the frequency of manifestation of pathological changes in muscle tissue (Kijowski and Konstanczak, 2009). In experiments on pigs, not only animal carcass weight but also the backfat thickness and the content of muscle tissue in a carcass (leanness) were assigned to risk factors (Minvielle et al., 2001).

Moreover, non-uniformity of hot carcasses by weight and leanness leads to problems with their chilling after slaughter, which, in turn, can be reflected in post-mortem formation of muscle tissue microstructure (Petracci and Cavani, 2012).

Therefore, significant differences in animal weight and leanness were undesirable as they could influence the development of myopathic changes in muscle tissue, “blurring” the picture of an effect of technological stress and adaptogens. It was noted in our experiment that control group 2 (C+) showed the lowest value of hot carcass weight, and experimental group 3 (C+Se) was distinguished by the lowest values of live weight beginning from the end of the 1st period of fattening up to slaughter. However, firstly, these differences in groups were not statistically significant; secondly, all carcasses obtained as a result of the slaughter of pigs from the control and experimental groups were assigned by weight and backfat thickness to the second category according to the existing Russian standard (GOST 31476, 2012). It was noted in our experiment that control group 2 (C+) showed the lowest value of hot carcass weight, and experimental group 3 (C+Se) was distinguished by the lowest values of live weight beginning from the end of the 1st period of fattening up to slaughter. However, firstly, these differences in groups were not statistically significant; secondly, all carcasses obtained as a result of the slaughter of pigs from the control and experimental groups were assigned by weight and backfat thickness to the second category according to the existing Russian standard (GOST 31476, 2012). The frequency of myopathy manifestation can be linked to reduced pH values in chilled pork. However, many researchers paid attention to the fact that there is a difference between meat with the signs of PSE and meat with the signs of myopathy (Minvielle et al., 2001;

Minvielle et al., 2001). The obtained data suggest that the carcasses from groups 1, 2, 3, and 4 did not display statistically significant differences in the average pH₂₄ value (Table 3). Moreover, analysis of variance using the Fisher–Snedecor test (F-distribution at $p = 0.05$, degrees of freedom: 3 and 35) confirmed the invalidity of the hypothesis about the presence of differences in the control and experimental groups by the pH₂₄ value ($f_{observed} = 2.00$, $f_{critical} = 2.92 \Rightarrow f_{observed} < f_{kp}$).

Quite low pH₂₄ values (5.41, 5.40, 5.49 and 5.47) in groups 1, 2, 3, and 4, respectively, were explained by stress in animals directly at slaughter (peculiarities of “peasant slaughter”) and indicated distinct manifestation of PSE properties in all meat samples. With that, a trend towards less pronounced PSE properties was observed in the experimental groups that received adaptogens compared with the control. Verification of this statistical hypothesis (at $p = 0.05$, degrees of freedom: 1 and 35) showed its validity ($f_{observed} = 5.90$, $f_{critical} = 4.17 \Rightarrow f_{observed} > f_{critical}$). This conclusion is in agreement with the results of many studies, which found a positive effect of organic selenium (Mahan, Cline, and Richert, 1999; Mateo et al., 2007; Li et al., 2011; Calvo et al., 2016; Calvo et al., 2017; Jiang et al., 2017) and DHQ (Vlahova-Vangelova et al., 2019) on the ultimate pH value of pork. The mean values of MHC for control groups 1 and 2, and experimental groups 3 and 4 were 62.61%, 67.05%, 69.18%, and 69.49%, respectively (Table 3). The differences in MHC between groups were confirmed statistically using the Fisher–Snedecor test (F-distribution at $p = 0.05$, degrees of freedom: 3 and 31; $f_{observed} = 3.04$, $f_{critical} = 2.92 \Rightarrow f_{observed} > f_{critical}$). Thus, the samples from all four groups were different regarding functional-technological properties in the absence of significant differences in muscle tissue pH in chilled pork.

The obtained results showed that the modelled technological stress during animal fattening and the use of adaptogens positively affected pork MHC. Similar conclusions regarding adaptogens were drawn by other studies (Mahan, Cline, and Richert, 1999; Mateo et al., 2007; Li et al., 2011; Calvo et al., 2016; Calvo et al., 2017; Jiang et al., 2017; Vlahova-Vangelova et al., 2019; Kremer, Stahly and Sebranek, 1998).

Differences in MHC in the absence of differences in pH₂₄ could be associated with different ratios of the main muscle fibre types in pork.

Table 3 Functional technological indices of pork muscle tissue.

Parameters	Values of the indices for groups			
	Control group 1, C-	Control group 2, C+	Experimental group 3, C+Se	Experimental group 4, C+DHQ
pH ₂₄ value*	5.41 ±0.06	5.40 ±0.08	5.49 ±0.14	5.47 ±0.09
MHC**, %	62.61 ±3.54	67.05 ±4.79	69.18 ±4.90	69.49 ±6.82

Note: * - (M ±m, n = 9); ** - (M ±m, n = 8).

Table 4 Ratio of different muscle fibre types in *M. longissimus dorsi*.

Item	Proportion of muscle fibres of the corresponding type, % of total amount / % of maximum value			
	Control group 1, C-	Control group 2, C+	Experimental group 3, C+Se	Experimental group 4, C+DHQ
White (II type)	78.4 ±0.8/99.7	78.6 ±0.8/100.0	78.5 ±0.8/99.9	78.6 ±0.8/100.0
Red (I type)	10.5 ±0.1/88.2	10.2 ±0.1/85.7	10.8 ±0.1/90.8	11.9 ±0.1/100.0
Intermediate types	11.1 ±0.1/99.1	11.2 ±0.1/100.0	10.7 ±0.1/95.5	10.2 ±0.1/91.1

Note: (M ±m, n = 5)

Table 5 Results of microstructural investigations of muscle tissue samples from *M. longissimus dorsi*.

Item	Control group 1, C-	Control group 2, C+	Experimental group 3, C+Se	Experimental group 4, C+DHQ
Morphometrical characteristics of muscle tissue				
Min sarcomere length, µm	1.4 – 1.8	1.7 – 2.1	1.5 – 2.0	1.5 – 1.9
Max sarcomere length, µm	1.7 – 2.0	1.9 – 2.3	1.7 – 2.5	2.0
Min perimysium thickness, µm	10.0	20.0	20.0	20.0
Max perimysium thickness, µm	20.0	50.0	50.0 – 55.0	35.0 – 40.0
Min thickness of fat tissue layers, µm	40.0 – 70.0	50.0 – 70.0	60.0 – 70.0	50.0 – 70.0
Max thickness of fat tissue layers, µm	130.0 – 200.0	170.0 – 200.0	200.0 – 330.0	200.0 – 240.0
Mean diameter of muscle fibres, µm	44.5 – 48.6	45.2 – 47.8	45.7 – 48.5	45.8 – 47.3
Signs of myopathy in white muscle fibres				
Min fibre diameter in nodes of contraction, µm	60.0	80.0	80.0	80.0
Max fibre diameter in nodes of contraction, µm	80.0	100.0	100.0	100.0
Max length of the supercontraction band, µm	800.0	400.0	800.0	300.0 – 350.0
General conclusion				
Pathological changes	Myopathy	Moderate myopathy	Myopathy	Moderate myopathy

Note: (n = 5)

Muscle fibres are dynamic structures, which can change from one type to another under certain conditions (Listrat et al., 2016).

The proportions of type I fibres (red, slow, oxidative), type II fibres (white, fast, glycolytic) and intermediate types depends on many factors, including muscle function, animal species, breed, age, physical activity, ambient temperature, nutritional and so on.

It is known that porcine skeletal muscles, especially in fast-growing hybrids, show a tendency towards an increased content of type II muscle fibres (Lefaucheur et al., 1998). With that, several studies show that destructive changes in muscle tissue, including the development of myopathy and meat quality deterioration, are linked with the prevalence of type II fibres (Realini et al., 2013; Kim et al., 2018; Lee et al., 2012). The highest content of type II fibres is typical for *M. longissimus dorsi*. In this context, it is recommended that this muscle be sent for histological investigations to

diagnose myopathic conditions in pigs (Realini et al., 2013; Cooper and Valentine, 2015).

The proportion of type II (glycolytic) fibres influences a tendency in meat tissue towards post-mortem development of nodes and bands of hypercontraction (so-called “giant fibres”), which are formed during the pig’s lifetime and directly linked with poor quality meat (Kim et al., 2018; Schubert-Schoppmeyer et al., 2008).

In our experiment, microstructural investigations of *M. l. dorsi* (Table 4) showed that the ratio of the main muscle fibre types was within the range of literature data (78.4% to 78.6%). According to the results of similar studies (Listrat et al., 2016; Sales and Kotrba, 2013), the proportion of white fibres in *m. L. dorsi* of fast-growing hybrids can be up to 90%. As a rule, red and intermediate fibres on average account for 10% each. Our results are in agreement with these tendencies.

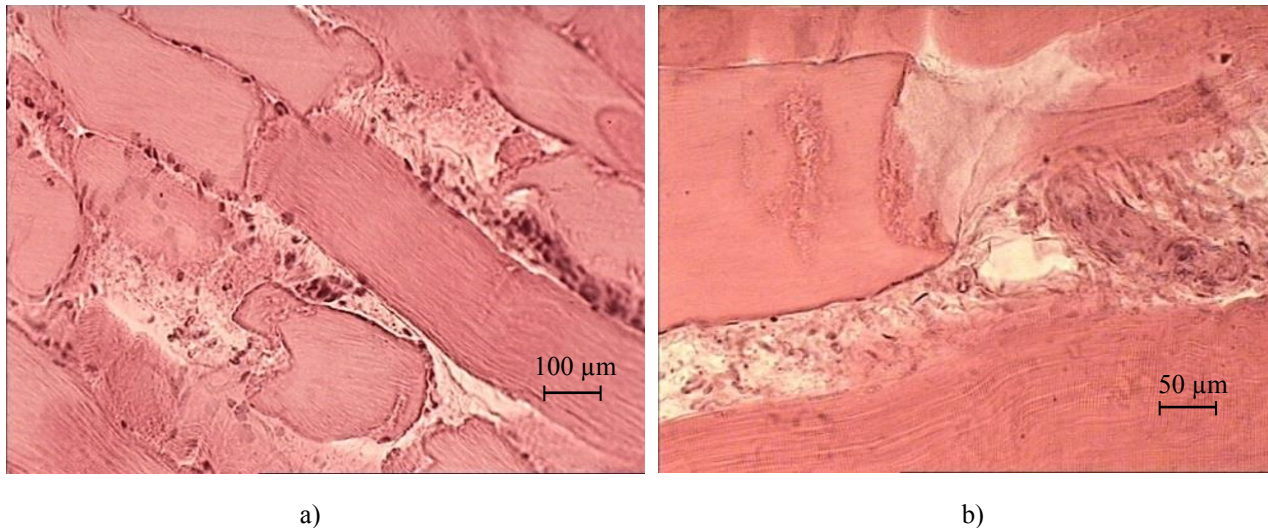


Figure 1 Microstructure of muscle tissue with signs of myopathy. Note: Longitudinal section. Non-uniform cross-striation (samples from groups 1 and 3). a) – nodes and bands of supercontraction with ruptures of myofibrils with a length of up to 800 µm. b) – formation of fine-grained protein mass at the sites of muscle tissue destruction as a result of damage to the sarcolemma integrity.

As follows from the data in table 4, some differences in the content of red and intermediate fibres were observed between the samples from the control and experimental groups. For example, the samples from experimental group 4 were characterized by the highest content of red fibres (11.9%) and the lowest content of intermediate fibres (10.2%), which likely had a positive effect on the functional-technological characteristics of muscle tissue but do not fully explain them.

During microstructural investigations of sections stained with haematoxylin and eosin, the complex of morphometrical indices of muscle tissue was determined, including the characteristics of white muscle fibres (Table 5).

Minimum and maximum sarcomere length in samples of *m. Longissimus dorsi* from animals of all groups were characterized by a rather wide range of values. The most stable sarcomere sizes were observed in sections of the samples from group 4. All samples from carcasses of animals subjected to the stress factor (groups 2, 3 and 4) showed a tendency towards an increase in the thickness of the connective tissue layers (perimysium) and an increase in the thickness of the fat tissue layers. The latter tendency was most pronounced in the muscle tissue samples from animal carcasses of groups 3 and 4, which received adaptogens. The mean diameter of muscle fibres without signs of supercontraction in the samples of all groups was quite uniform and corresponded to the animal species and characteristics of the muscle that was chosen for investigation (*M. longissimus dorsi*).

At the same time, all samples showed signs of myopathy, which were manifested in peculiarities of generative changes in the structure of white muscle fibres. In the samples of control group 1, the diameter of nodes of contraction in the cross-sections was 60 to 80 µm. The bands of super contraction with multiple ruptures of myofibrils with a length of up to 800 µm with non-uniform cross-striation were observed in the longitudinal sections. The fine-grained protein mass was found at the sites of

muscle fibre destruction as a result of damage to the sarcolemma integrity.

Similar changes in the structure of white muscle fibres were also observed in the muscle tissue samples from the third group of animals, which received selenium. With that, the fibre diameter in the nodes of contraction was 80 to 100 µm.

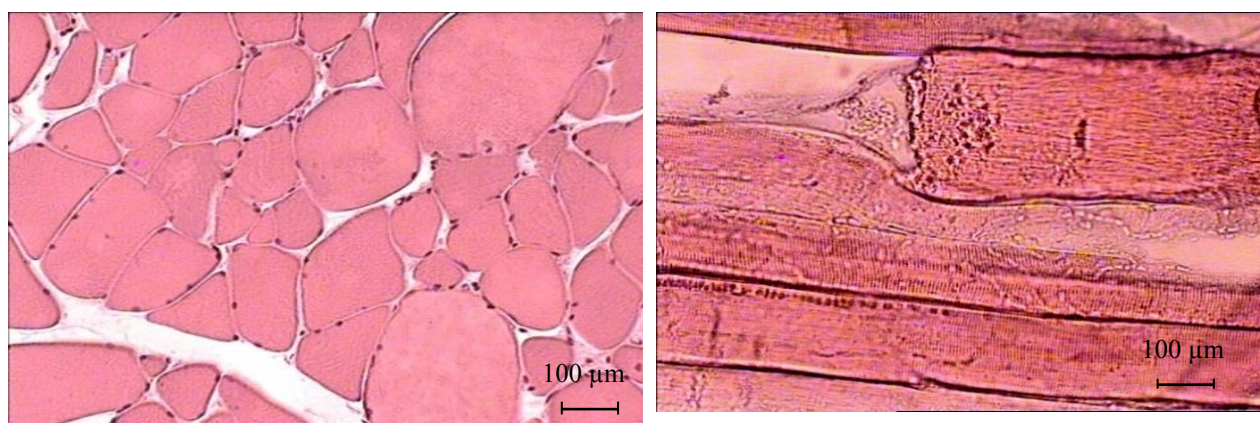
The revealed pathological changes in the samples from groups 1 and 3 corresponded to the picture of myopathy (Figure 1 and Figure 2).

In the samples from group 2, upon similar sizes of white fibres in nodes of contraction (80 to 100 µm), the bands of super contraction with the lengths of not more than 400 µm were observed in the longitudinal sections, which allowed diagnosis of only moderate myopathy.

In the samples from group 4, bands of super contraction had a maximum length of not more than 350 µm, which undoubtedly facilitated better retention of pork functional-technological properties and partly explained the high results obtained in MHC measurement. On the other hand, a higher proportion of red fibres was found in the samples from group 4, which can also facilitate higher MHC values. The pathological changes revealed in the samples from groups 2 and 4 corresponded to the picture of moderate myopathy (Figure 3).

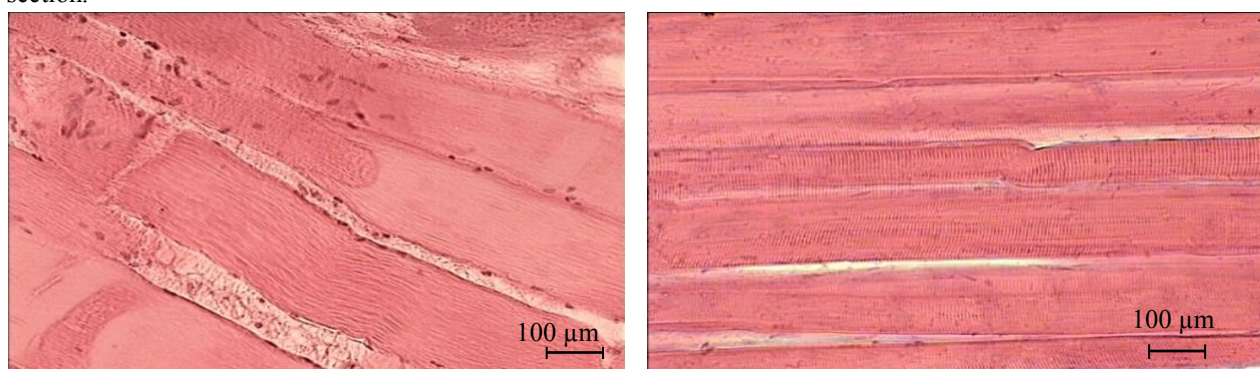
A comparison of the results of microstructural investigations showed that supplementation of animal diets with DHQ had a greater effect on the condition of pork muscle tissue than the use of selenium in the diet. These results are in good agreement with the data of other researchers.

For example, it was concluded in three studies (Calvo et al., 2016; Falk et al., 2018) that the addition of selenium, irrespective of the source, in the diet did not influence the muscle tissue condition. However, it was suggested that during meat aging selenium could enhance destructive changes in muscle tissue caused by an increase in the activity of tissue enzymes. It is necessary to note that data on the effect of DHQ on the condition of pork muscle tissue was not found in the available scientific literature.



a) b)

Figure 2 Microstructure of muscle tissue with signs of myopathy (samples from groups 1 and 3). Note: a) – nodes of supercontraction with the round shape in the cross section and a diameter more 100 μm ; b) – nodes of supercontraction of myofibrils with pronounced longitudinal striation and ruptures of the sarcolemma more 100 μm in the longitudinal section.



a) b)

Figure 3 Microstructure of muscle tissue with signs of moderate myopathy (samples from groups 2 and 4). Longitudinal section. Note: a) – nodes of supercontraction of myofibrils with ruptures of sarcolemma and pronounced longitudinal striation with a length near 400 μm . Non-uniform cross-striation; b) – transversal bands of myofibril supercontraction in the muscle fibre structure. Muscle fibres with the straight shape and pronounced cross-striation lay loosely in relation to each other.

However, the results of recent studies on laboratory animals have shown that long-term addition of quercetin (analogue of DHQ) reduces muscle damage (Hollinger et al., 2015; Mukai et al., 2016).

Therefore, the combination of modelled technological stress during animal fattening with the addition of DHQ into a diet partly leveled pork quality deterioration caused by an effect of pre-slaughter stress in animals, allowing the production of meat with moderate signs of myopathy and, consequently, with higher functional-technological characteristics.

CONCLUSION

A decrease in pork functional-technological characteristics, such as MHC, is not always conditioned to the full extent by the pH value of muscle tissue. The manifestation of myopathy, which is expressed in destructive changes in muscle tissue, is typical for fast-growing hybrid animals fattened under conditions of industrial enterprises. The results of this study showed that the presence of such modelled technological stress factors as the relocation of animals during fattening positively influenced the muscle tissue microstructure after slaughter.

At the same time, additional incorporation of DHQ as an adaptogen reduced the development of myopathy signs to a greater extent. DHQ showed better results in this regard than selenium as a distinct manifestation of destructive regions in muscle tissues was lower in the group of animals that received DHQ in the diet for a long time. In the future, accumulation and systemization of scientific data on the manifestation of pathological changes in the muscle tissue structure and methods for their prevention will allow more effective management of the process of lifetime formation of pork quality.

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