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# HISTOCHEMICAL ANALYSIS OF SKELETAL MUSCULAR TISSUES OF PIGS ACCORDING TO GENOTYPE MYF4**

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**Abstract:** The results of histochemical analysis of three muscles *m. triceps brachii* (MTB), *m. longissimus* thoracicus (MLT) and *m. rectus femoris* (MRF) of two groups of pigs created according to the genotypes *MYF 4* are presented. Determination of *MYF 4* genotypes was made by PCR method and for histochemical analysis was used 5 animals detected as homozygote *MYF 4-AA* type and 5 animals of heterozygote genotype myogenin-*AB* out of the total of 25 individual animals tested. The histochemical analysis proved that homozygotes *AA* have had bigger fat cells than heterozygotes *AB* in three studied muscles in average. The size of fat cells in MLT – 41.10μm or 38.50 μm respectively dominated in both groups of animals. Percentage surface representation of interstitial tissues was higher in the studied muscles of heterozygote *MYF 4-AB*. The volume of ligaments was the highest in MRF (3.80% or 3.90% respectively) in both groups (myogenin – *AA* and *AB*). The average thickness was of three studied muscles muscle fibres higher at homozygote genotype myogenin-*AA* than in heterozygote myogenin-*AB*. The thickest fibres in both genotypes were in MRF (88.60 μm, and 84.72 μm respectively) and the lowest in MTB (73.30 and 69.40 μm respectively). The highest values of muscle fibres thickness were detected in α-White fibres. Their percentage surface representation corresponded to this in all three types of muscles of both studied genotype myogenin groups.

**Key words**: pigs, striated muscles, histochemical analysis, detection of myogenin

# Introduction

Striated skeletal muscle is at the present a subject of intensive research as muscles representing one of the most important elements in human nutrition. The study of muscle growth and meat quality led to the increasing of interest in microscopic skeletal muscle structure of individual types of animals (*Ijačky et al., 1997; Ľahučký, 2003; Dransfield et al., 2003*). The relationship of qualitative and quantitative properties of skeletal muscles and final meat product is determined also by the growth changes of individual farm animals (*Uhrin, 1997*). At the end, the histochemical methods allowing us to follow and evaluate quality and quantity differences in the final product. It is clear that there are quantitative defined differences among the individual farm animals which are under genetic control of several genes. One of them is myogenin (*Ernst et al., 1994*), which is essential activator of myogenesis (*Rawls et al., 1997*). It is justifiable to observe and to quantify the existing differences in the same types of farm animals directly genetically dependent under supposition that the nutrition, the movement, the stabling and various other factors of the external environment are the same (*Chladek and Ingr, 2003)*.

# Material and methods

The Landrace breed of pigs was used for this experiment and they were reared in the standard conditions (age, nutrition, stabling etc.). The average weight of animals at the end of the experiment was 96.3 kg (+- 3 kg). Immediately after the slaughtering of 25 animals we took blood samples in order to be able to detect the genotypes of myogenin gene and we also took samples of three muscle types m. triceps brachii (MTB), m. longissimus thoracicus (MLT) and m. rectus femoris (MRF). These were immediately frozen in liquid nitrogen after the sampling. The genetic analyse of myogenin gene was made in the laboratory of genetic department by PCR and on the basis of the detection of myogenin genotypes we did the histochemical analyses of three muscle types (MTB, MLT, MRF) from five animals – homozygotes myogenin-AA and five animals- heterozygotes myogenin-AB. The frozen muscle samples were processed in the histochemical laboratory in cryostat when we cut the series of slices, width 10 μm at the temperature –18˚C. The slices were incubated for succinic dehydrogenase activity to (*Lojda and Papousek, 1978*) in order to be able to detect muscle fibre types. The next series of slices were dyed with hematoxylin-eosin and the third series of slices was stained by oil red "O" in order to detect the lipids. The morphometric methods according to (*Uhrin and Kulisek, 1980*) was used to detect the thickness and type of muscle fibres as well as percentage representation and ligaments. We used the lanameter to

measure the size of fat cells while enlarged 500x. The results were evaluated by common statistical methods.

## Results and discussion

*The analysis of fat tissues*

There is a certain hierarchy from the morphological point of view regarding the storage of neutral lipids in the cells of interstitial ligaments in the skeletal muscles depending on the endogenous and exogenous factors. In our study we detected the fat cells of various size and localisation in muscles in all three studied muscles in both genotypes (myogenin-AA and AB respectively). The histological and chemical evaluation was based on the fact that the external environment factors were the same. Therefore we are coming to the conclusion that the results and differences are influenced by the endogenous and physiological factors of fat storage in this particular tissue and they are not influenced by the external environment factors.

We detected in this experiment the fat cells of various localisations and size in the interstitial tissues in all three studied muscles of both genotypes (homozygotes AA and heterozygotes AB). We can say from the subjective evaluation of the samples that the fat cells were located in the majority in the interstitial tissues amongst the tertiary bundles of muscle fibres. As a rule they were grouped as a formation of 10-30 univacuole cells surrounding the bigger blood veins. They were separated from the bundles of muscle fibres by uninterrupted layer of perymisium internum.

The muscle division into smaller sub- bundles (secondary and primary bundles) causes the decrease in the numbers and the thickness of fat cells. We detected the isolated cells 1 to 3 with small diameter among the primary muscle bundles. The majority of them had multivacuole characteristics. The real and comparative characteristics were achieved on the basis of morphometric measurement of fat cell size. The highest average size value of fat cells in homozygotes myogenin–AA was in MLT - 41.10 μm. Slightly smaller was the size of fat cells in MTB - 39.70 μm. The fat cells in MRF were considerably smaller and their diameter was 36.70 μm. The minimal and maximal values show that the variation in the size of fat cells in the studied muscles was very constant (Table 1)

**Table 1. The size of fat cells in µm – homozygotes myogenin- AA**
**Tabela 1. Veličina ćelija masti u µm – homozigotni miogenin- AA**

| | X pr. | Min | Max | Dispersion/ Disperzija | Standard deviation/ Standardna dev. | V |
|---|---|---|---|---|---|---|
| MTB | 39,70 | 26,00 | 54,00 | 54,51 | 7,38 | 18,60 |
| MLT | 41,10 | 24,00 | 56,00 | 64,39 | 8,02 | 19,52 |
| MRF | 36,70 | 24,00 | 50,00 | 49,11 | 7,01 | 19,09 |

The size of fat cells in MLT - 38.50 µm was again prevailing in the animals detected as heterozygotes (AB). The smallest average thickness was in MTB - 34.85 µm and in MRF was slightly higher 35.70 µm. The thickness of muscle fibres in the three studied muscles in all these animals was smaller than with homozygotes (AA) but the variability was higher (Table 2).

**Table 2. The size of fat cells in µm – heterozygotes myogenin- AB**
**Tabela 2. Veličina ćelija masti u µm – heterozigotni miogenin- AB**

| | X pr. | min | max | Dispersion/ Disperzija | Standard deviation/ Standardna dev. | V |
|---|---|---|---|---|---|---|
| MTB | 34,85 | 20,00 | 54,00 | 86,18 | 9,28 | 26,64 |
| MLT | 38,50 | 20,00 | 54,00 | 85,15 | 9,23 | 23,97 |
| MRF | 35,70 | 24,00 | 52,00 | 50,71 | 7,12 | 19,95 |

*Muscle fibre analysis:*

We paid attention to the average thickness of muscle fibres and the average thickness of the individual types of the muscle fibres while studying the muscles of both groups of genotypes. We studied also the surface proportion and the percentage proportion of interstitial tissues and the percentage proportion of the individual types of muscle fibres.

The histochemical analysis showed that the average thickness of muscle fibres varies among the genetically detected groups. But the thickness tendency in muscles is the same. Generally we can say the homozygote myogenin-AA showed higher average values of thickness of muscle fibres in all three muscle types (MTB, MLT, MRF). The homozygote AA had the highest average thickness in MRF - 88.60 µm and the lowest in MTB - 73.30 µm. The fibres had the average thickness of 79.03 µm in MLT. The heterozygotes myogenin-AB

had the same tendency of thickness in the direction of MRF (84.72 μm) - MLT (75.77 μm) - MTB ( 69.40 μm) (Tables 3, 4).

While evaluating the average size of muscle fibre types, it is possible to state that in all studied muscles, both genotype groups (AA and AB) the α-White fibres are dominant. We detected the highest values of thickness in MRF both genotypes (90.58 μm respectively 86.98 μm) The values were higher in all the muscles than the average size of muscle fibres.

We detected the lowest values of muscle fibres with β-Red fibres again with all the muscles of both genotype groups. Their average thickness was at its lowest in MTB (71.36, respectively 66.40 μm) in homozygote myogenin-AA and heterozygote myogenin-AB. The intermediary α-White had the mean values and their size more or less corresponded to the average size of muscle fibres (Tables 3, 4).

**Table 3. The average thickness of muscle fibres and their types–homozygotes myogenin–AA**
**Tabela 3. Prosečna debljina mišićnih tkiva i njihovi tipovi– homozigotni miogenin- AA**

| | MTB | | | | MLT | | | | MRF | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B-Red | A-Red | A-White | Total | B-Red | A-Red | A-White | Total | B-Red | A-Red | A-White | Total |
| x average/ x prosek | 71,36 | 73,24 | 75,31 | 73,3 | 79,32 | 78,81 | 78,95 | 79,03 | 84,61 | 84,61 | 90,62 | 88,6 |
| dispersion /disperzija | 7,66 | 7,61 | 7,26 | | 8,38 | 10,24 | 9,42 | | 12,68 | 121,5 | 12,53 | |
| Standard/s tandard | 2,77 | 2,76 | 2,69 | | 2,89 | 3,2 | 3,07 | | 3,56 | 11,02 | 3,54 | |
| Variable k./promen .k. | 3,77 | 3,77 | 3,68 | | 3,65 | 4,06 | 3,89 | | 3,93 | 13,03 | 3,91 | |

**Table 4. The average thickness of muscle fibres and types - heterozygotes myogenin- AB**
**Tabela 4. Prosečna debljina mišićnih tkiva i njihovi tipovi– heterozigotni miogenin- AB**

| | MTB | | | | MLT | | | | MRF | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B-Red | A-Red | A-White | Total | B-Red | A-Red | A-White | Total | B-Red | A-Red | A-White | Total |
| x average/ x prosek | 66,4 | 69,41 | 72,4 | 69,4 | 73,76 | 75,78 | 77,76 | 75,77 | 82,18 | 84,99 | 86,98 | 84,72 |
| dispersion/ disperzija | 2,9 | 2,9 | 2,91 | | 2,17 | 2,14 | 2,16 | | 5,15 | 3,55 | 3,52 | |
| Standard/st andard | 1,7 | 1,7 | 1,71 | | 1,47 | 1,46 | 1,47 | | 2,27 | 1,88 | 1,88 | |
| Variable k./promen. k. | 2,45 | 2,45 | 2,46 | | 1,94 | 1,93 | 1,94 | | 2,7 | 2,22 | 2,21 | |

While studying the surface proportion of the individual fibres and interstitial tissues in percentages we learned about the additional analysis of muscle structure. It was proven again that the surface proportion of α-White fibres is at its highest in all muscle groups in both genotypes myogenin. The results show that the surface proportion of those fibres in difference to their size is at its lowest values in MRF in both genotype groups AA and AB (40% respectively 46.60%). This results in that β-Red a α-Red fibres represent proportionally more percents than α-White even thorough are the thickest ones in their diameter (Tables 5, 6).

**Table 5. Surface proportion of individual fibres and ligaments in percentages homozygote myogenin-AA**
**Tabela 5. Odnos površine individualnih tkiva i ligamenata u procentima homozigotnog miogenina –AA**

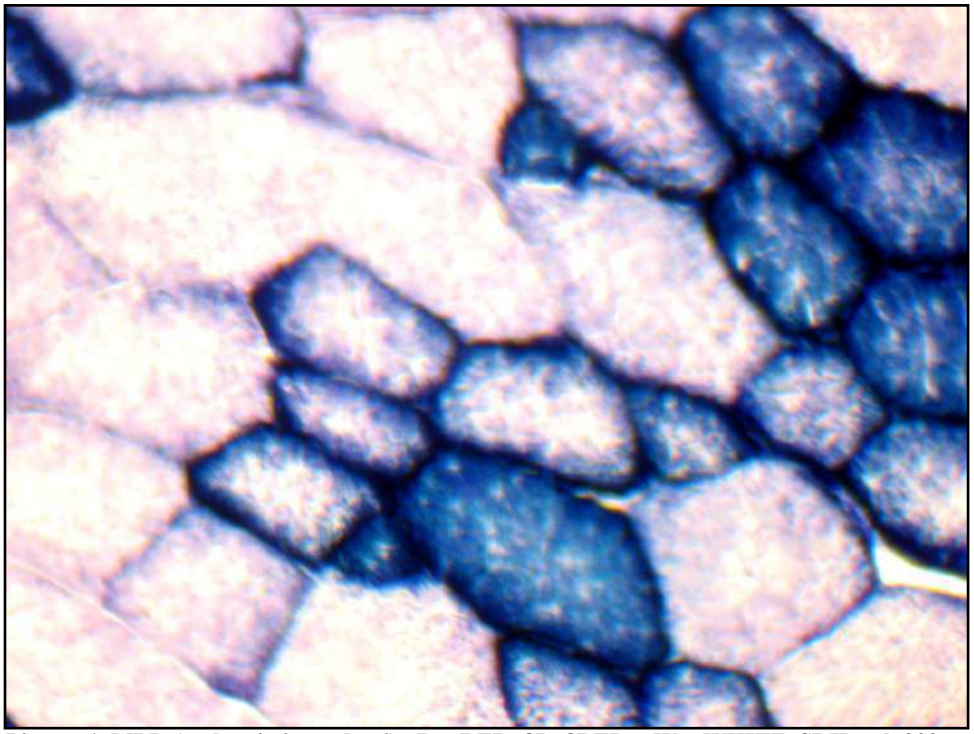
| | MTB | | | | MLT | | | | MRF | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B-Red | A-Red | A-White | Iv | B-Red | A-Red | A-White | Iv | B-Red | A-Red | A-White | Iv |
| X average/ X prosek | 25,97 | 14,97 | 55,66 | 2,4 | 17,68 | 27 | 51,8 | 3,51 | 24,68 | 31,52 | 40 | 3,8 |
| Dispersion/ disp. | 4,78 | 3,85 | 8,87 | 0,07 | 2,07 | 2,53 | 4,46 | 0,08 | 2,17 | 45,78 | 34,23 | 0,24 |
| Standard/sta nd. | 2,19 | 1,96 | 2,98 | 0,25 | 1,44 | 1,59 | 2,11 | 0,28 | 3,41 | 6,77 | 5,85 | 0,48 |
| variable k./prom.k. | 8,42 | 13,1 | 5,26 | 10,62 | 8,14 | 5,89 | 4,08 | 7,91 | 13,81 | 21,47 | 14,63 | 12,76 |

| | MTB | | | | MLT | | | | MRF | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B-Red | A-Red | A-White | Iv | B-Red | A-Red | A-White | Iv | B-Red | A-Red | A-White | Iv |
| X average/ X prosek | 28,73 | 13,26 | 54,4 | 3,61 | 25,06 | 15,44 | 55,93 | 3,58 | 27,63 | 21,87 | 46,6 | 3,9 |
| Dispersion/ disp. | 2,17 | 0,96 | 2,44 | 0,14 | 2,82 | 3,08 | 1,12 | 0,08 | 5,21 | 5,11 | 5,34 | 0,14 |
| Standard/stand. | 1,47 | 0,98 | 1,56 | 0,37 | 1,68 | 1,76 | 1,06 | 0,29 | 6,7 | 2,26 | 2,31 | 0,38 |
| variable k./prom.k. | 5,21 | 7,4 | 2,87 | 10,36 | 2,28 | 11,37 | 1,89 | 8,04 | 8,26 | 10,33 | 4,96 | 9,62 |

**Table 6. Surface proportion of individual fibres and ligaments – heterozygote myogenin-AB**
**Tabela 6. Odnos površine individualnih tkiva i ligamenata u procentima heterozigotnog miogenina-AB**

# Conclusion

In spite of the fact that there are not enough literature resources about the histochemical muscle analysis of various pig breeds in various breeding conditions there is a shortage of genotype data of candidate genes associated with the development of muscle in the individual animal types. We detected the genotypes myogenin and we divided the sample animals into two groups-homozygotes myogenin-AA and heterozygotes myogenin-AB. The histological and morphological analysis showed that there are better qualitative signs of muscle fibres in relation to the quality of the final product – meat in homozygotes myogenin-AA regarding the studied muscles.



**Picture 1. MLD (m. longissimus dorsi) αR–αRED, βR–βRED, αW–αWHITE. SDH. enl. 200x**
**Slika 1. MLD (m. longissimus dorsi) αR–αRED, βR–βRED, αW–αWHITE. SDH. enl. 200x**

# Histohemijska analiza skeletnog mišićnog tkiva svinja prema genotipu MYF4

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## Rezime

Rezultati histohemijske analize tri mišića *m. triceps brachii* (MTB), *m. longissimus* thoracicus (MLT) i *m. rectus femoris* (MRF) svinja raspoređenih u dve grupe prema genotipovima *MYF 4* su predstavljeni u radu. Određivanje *MYF 4* genotipova je urađeno PCR metodom i za histohemijsku analizu

korišćeno je 5 grla otkrivenih kao homozigotni *MYF 4-AA* tip i 5 grla heterozigotnog genotipa miogenin-*AB* od 25 pojedinačnih grla koja su testirana. Histohemijska analiza je pokazala da homozigoti *AA* su imali veće ćelije masti od heterozigota *AB* kod tri ispitivana mišića u proseku. Veličina ćelija masti u MLT-u – 41.10μm i 38.50 μm respektivno je bila dominantna u obe grupe životinja. Procentualno izražena površina intersticijalnog tkiva je bila veća u ispitivanim mišićima heterozigota *MYF 4-AB*. Obim ligamenata je bio najveći u MRF-u (3.80% ili 3.90% respektivno) u obe grupe (miogenin – *AA* i *AB*). Prosečna debljina mišićnih vlakana u tri ispitivana mišića je bila veća kod homozigotnog genotipa miogenin-*AA* nego kod heterozigotnog miogenin-*AB*. Najdeblja vlakna u oba genotipa su utvrđena kod mišića MRF (88.60 μm, i 84.72 μm respektivno) a najmanja debljina kod mišića MTB (73.30 i 69.40 μm respektivno). Najveće vrednosti za debljinu mišićnih vlakana su utvrđene kod α-belih vlakana. Njihova procentualna zastupljenost je odgovarala mišićima sva tri tipa oba ispitivana genotip miogenin grupa.

**Ključne reči**: svinje, mišići, histohemijska analiza, određivanje miogenina

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