

GENETICAL INFLUENCE ON HISTOLOGICAL STRUCTURE AND CHEMICAL COMPOSITION OF MUSCULAR TISSUE IN SHEEP

Vladimir Ivanovich TRUKHACHEV^{1*}, Marina Ivanovna SELIONOVA¹, Irina Ivanovna DMITRIK², Milan P. PETROVIC³, Violeta Caro PETROVIC³, Dragana RUZIC-MUSLIC³, Nevena MAKSIMOVIC³

¹Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, Moscow, 127550, Russia

²FGBNU All-Russian Research Institute of Sheep and Goat breeding, 355015, Stavropol, Russia

³Institute for Animal Husbandry, Zemun, Belgrade, Serbia

Trukhachev V. I., M. I. Selionova, I. I. Dmitrik, M.P. Petrovic, V. Caro Petrovic, D. Ruzic-Muslic, N. Maksimovic (2021). *Genetical influence on histological structure and chemical composition of muscular tissue in sheep*. - Genetika, Vol 53, No.1, 209-218.

The effect of different genotypes kept in the same feeding level on microstructure and chemical composition of muscular tissue (musculus longissimus dorsi) of sheep has studied. The study aims to see how the gene combination of different breeds influences the mentioned problem. It was revealed that sheep muscular tissue obtained by crossing the North-Caucasian breeds (NC) and Texel (T) was characterized by a larger quantity of muscular fibres by 4.73 % and 10.14% ($P < 0.05$), and a smaller diameter by 5.65 % and 18.6% ($P < 0.05$), a higher evaluation of marbling by 5.2 and 8.3 points, less connective tissue content by 0.8 and 1.3 absolute percentage compared to thoroughbred North-Caucasian and from crossing North-Caucasian with Poll Dorset (PD). It showed that meat obtained from crossing animals of the T×NC variant is more delicate, succulent, and has a total higher quality and consumer properties.

The results of different crossing indicate the reasonability to use morphological characteristics of muscular tissue on the histological level when the quality of sheep slaughtering performance had evaluated.

Keywords: genotype, crossbreeding, sheep slaughtering performance, meat quality, micro-structural analysis, chemical composition

Corresponding author: Vladimir Ivanovich Trukhachev Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, Moscow, 127550, Russia E-mail: m_selin@mail.ru

INTRODUCTION

The genetic influence on the quality of muscle tissue and thus the quality of sheep meat has been increasingly the subject of scientific research. For example, one of the new candidate genes that define the productive traits of sheep is MEF2B protein affecting myostatin production and expression of genes responsible for growth skeletal muscle fibers (TRUKHACHEV *et al.*, 2016).

Therefore, genetic contribution to variation in meat quality must be seriously considered and understood in the livestock industry, which strives for high product quality (WARNER *et al.*, 2010). Muscles have different functional properties related to evolutionary requirements for body function, reproduction and survival rather than their eating qualities and to serve these functions they vary greatly within the animal, not only in their fibre and protein properties but also in other, associated compositional characteristics (CLARKE *et al.*, 1996). Muscle mostly studied with respect to meat production, represents one largest protein reservoirs of the body (JAN *et al.*, 2016).

The selective breeding programs directing to increase the productivity and profitability of the sheep meat industry use elite, progeny tested sires. The broad genetic traits of primary interest in the progeny of these sires include skeletal muscle yield, fat content, eating quality, and reproductive efficiency (TELLAM *et al.*, 2012). As a whole, varied factors directly or indirectly affect the quality characteristics of meat (DE LIMA JUNIOR *et al.*, 2016), that the determination and analysis of marbling plays an important role in the quality evaluation of meat, which is of great importance to the meat industry (CHENG *et al.*, 2015).

Modern domestic and world experience confirms economical reasonable to obtain high-quality mutton of young sheep animals (GADZHIEV, 2010). In this connection, the interest in the questions to form high slaughtering performance of sheep and especially its qualitative characteristics has been enhance now.

Investigations concerned to study qualitative characteristics of muscular tissue on the micro-structural level is extremely insufficient depending on the degree of fatness and genetical aspect (DMITRIK and OVCHINNIKOVA, 2013; LUSHNIKOV *et al.*, 2013).

At the same time, the histological analysis allows to have a specific characteristic of raw meat products on the level of muscular fibres, namely, the degree of their development in different muscles, the number and diameter per unit area, the quantitative and qualitative parameters and architectonics of muscular fibre layers which determine so-called "marbling" and partly meat succulence. The content and character of connective tissue distribution both between individual muscular fibres and integral muscular bundles is no less important in terms of mutton qualitative characteristics, namely, the formation such characteristic as meat tenderness (HULLAND, 1981; HONIKEL, 1987; SELIONOVA *et al.*, 2014).

The wide application of precise but averaged investigation methods (chemical, biochemical, etc.) allows getting only part of the necessary information about meat quality. The method of micro-structural histological analysis is a direct method to determine the condition of raw material and meat products, which presents another but no less valuable part of the information. Micro-structural investigations allow judging both the product structure on the whole and changes taking place in some parts and components of studied objects. Micro-structural methods significantly increase findings received by means of other methods and allow

finding actually small changes in tissue structures which affect meat quality (LUSHNIKOV *et al.*, 2013; KHVYLYA and DONSKOVA, 2006; KUSCHFELD, 1986; DE MACEDO *et al.*, 2000).

Genetic evaluation for lamb production has enabled impressive genetic gains (HOPKINS and MORTIMER, 2012). Genetic improvement of sheep is the basis for sustainable development of this branch of animal husbandry (ZINOVIEVA *et al.*, 2015). The genetic aspect has a significant impact on the yield and quality of sheep meat. Therefore, cross-breeding is applied to use the heterosis effect (CARO PETROVIC *et al.*, 2015; PETROVIC and PANTELLIC, 2015; PETROVIC *et al.*, 2018). Most researchers investigate the dynamics of lamb body weight development. There is very little research regarding the genetic impact through cross-breeding on histological muscle structure, which has a major impact on the quality of lamb meat. This paper aims to see how the gene combination of different breeds influences the mentioned problem.

MATERIALS AND METHODS

The samples of the longest back muscle (musculus longissimus dorsi) of the thoroughbred ram-lambs of the North-Caucasian mutton-wool breed (NC) and ram-lambs obtained from two crossing variants with Texel (T×NC) and Poll Dorset (PD×NC) at nine month age have studied connected with the investigation of the histological structure of muscular tissue depending on the genotype. The investigation conducted at the experimental farm of the All-Russian Scientific-Research Institute of Sheep and Goat Breeding, located at North Caucasus region. Summer temperatures vary from +3,1 to +28°C and winter vary from -1,7 to -28,3°C;). The groups in 15 each kept under the equal feeding conditions to have standard animals with a live weight of 34.8, 35.7, 36.0 kg, and a carcass weight of 15.5, 15.9, 16.0 kg respectively according to their age.

Slaughter of the experimental animals and evaluation of the obtained carcasses were carried out in accordance with Russian GOST № 52843-2007 “Sheep and goats for slaughter. Mutton, lamb and goat meat in carcass”. The samples of the longest back muscle (musculus longissimus dorsi) have taken at the level of the 10th -12th rib of the cooled right half-carcass, which were labelled and fixed in 10% formalin solution. The preparation of muscular tissue (spirituous, celloidin techniques, and block hardening), histological sections and their staining have performed according to the standard protocol described in the Guideline “The method of the histological evaluation of the qualitative characteristics of sheep slaughtering performance with regard to the tissue morphologic structure” (DMITRIK *et al.*, 2010).

The sections of muscular tissue to 8-10 microns in thickness obtained on the sledge microtome and have stained by hematoxylin and eosin. The muscular fibre diameters have measured at a magnification in 500 times in three fields of view of the microscope using an ocular-micrometer MOS-1-16x. The slides observed using a binocular microscope MIKMED-6 TU 9443-168-07502348-2005 with “MCview” software. The histological results had described and documented.

To measure an area of the muscular eye, the longest back muscle (musculus longissimus dorsi) had cut between the last thoracic vertebrae. This cross-section cut had put over a parchment paper, and muscle contours had transferred. The contour area (cm²) has determined by multiplying the length by its width. Chemical composition of muscular tissue was performed according to Russian standard GOST 23392-2016.

The biometric processing of received materials was carried out using BIOSTAT and MS Office software package.

RESULTS

The results of microstructural meat characteristics of crosses at 9 months of age are shown in table 1.

Table 1. Microstructural meat characteristics of crosses at 9 months of age

Trait	Breed genotypes, LSM±SE, n=15			P value
	NC	TxNC	PDxNC	
Number of muscular fibres on mm ²	392.8±9.2 ^a	412.3±13.6 ^b	370.5±14.3 ^a	*
Diameter of muscular fibres, micron	28.3±0.7 ^a	26.7±1.7 ^a	32.8±1.9 ^a	-
«Marbling », evaluation, point	33.5±0.9 ^a	38.7±1.2 ^b	30.4±0.8 ^a	**
Connective tissue content, %	8.9 ^a	8.1 ^b	9.4 ^a	***
Meatness (eye muscle area, cm ²)	13.5±2.3 ^a	16.7±2.0 ^a	15.2±4.0 ^a	-

A-B Means with similar letters in each sub-class within a row do not differ from another at *p<0,05* p<0,01

The analysis of the received results (Table 1) testifies that sheep meat produced from animals of the crossing variant T×NC is characterized by many muscular fibres per mm² by 4.73%, 10.14% (P<0.05), and a smaller diameter by 5.65 % and 18.6% compared with thoroughbred (NC×NC) and PD×NC respectively (Images 1-3).

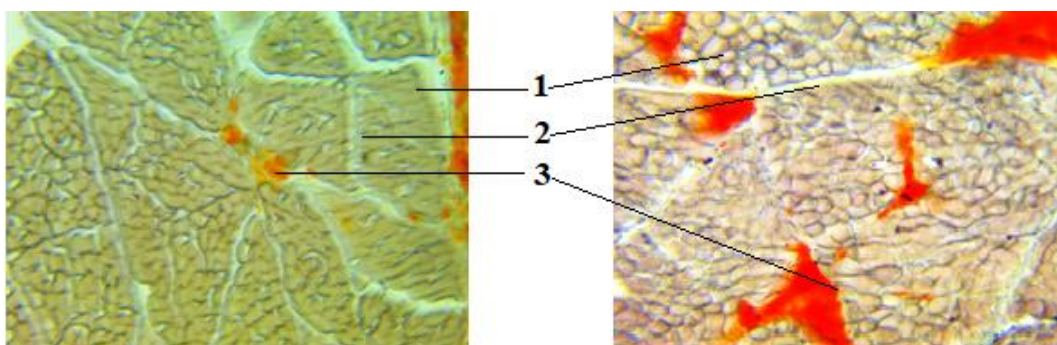


Image 1 The histological section of the muscular tissue of the longest back muscle of the ram-lambs of NCx NC (stained by hymatoxylin-eosin, magnification x 500 times)

Image 2 The histological section of the muscular tissue of the longest back muscle of the ram lambs of Tx NC (stained by hymatoxylin-eosin, magnification x 500 times)

A great number of fatty layers between fibres and between bundle fibres were observed to TxNC due to a higher evaluation of marbling up to 5.2 and 8.3 points respectively with thoroughbred (NC×NC) and PD×NC, (P<0.05) (Images 1-3).

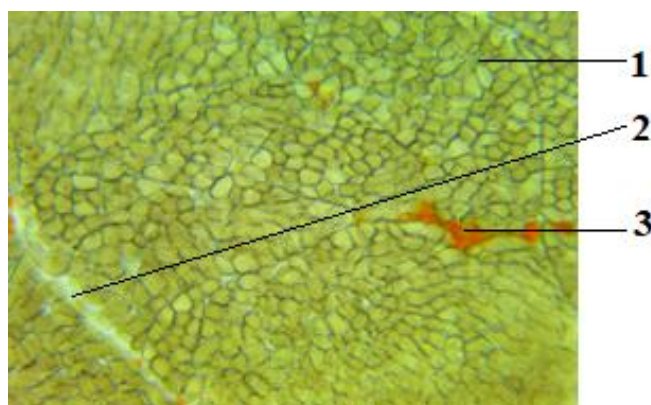


Image 3. The histological section of the muscular tissue of longest back muscle of the ram-lambs of PDxNC (stained by hymatoxylin-eosin, magnification x 500 times)

1 – diameter of the muscular fibre; 2 –connective tissue; 3 – fatty interfibrinous and interfascicular inclusions

In addition, a lower amount of connective tissue (Table 1) by 0.8 and 1.3 absolute percentage was in the longest back muscle (musculus longissimus dorsi) compared to thoroughbred (NCxNC) and PDxNC respectively (Images 1-3).

One of the indicators characterizing slaughtering performance is an eye muscle area (Table 1). This indicator was higher by 19.16% ($P < 0.01$) with TxNC animals as compared with thoroughbred (NCxNC) and by 8.92% ($P < 0.05$) with PDxNC.

The ram-lambs of the PDxNC genotype had coarse-fibre muscular tissue with a diameter of 32.8 microns, and the greatest inclusion of connective tissue – 9.4% (Table 1).

Table 2. Chemical composition of sheep meat of crosses (9 months)

Content %	Groups		
	NC	TxNC	PDxNC
Moisture	74.4±0.78	73.2±0.85	73.3±0.79
Dry matter	25.6±0.26	26.7±0.29	26.8±0.32
Fat	4.7±0.23	5.8±0.21	5.7±0.19
Ash	1.3±0.03	1.2±0.08	1.2±0.05
Protein	19.7±0.30	19.8±0.29	19.9±0.30
Calorie content 1 kg of flesh, kcal	1343.7±42.3	1443.0±4.2	1446.3±41.8

The conducted chemical analysis of meat (Table 2) has not demonstrated significant differences between genotypes. There was a tendency to more dry matter content and fat by 1.1 and 0.1 absolute percentage in animal meat of the TxNC genotype and less moisture amount by 1.2 and 0.1 absolute percentages as compared with thoroughbred sheep and PDxNC. Thoroughbred (NCxNC) showed higher ash content for 0.1 both for TxNC and PDxNC. On the other hand, PDxNC showed highest in protein and calorie content per 1 kg of flesh.

However, it points to the fact that meat obtained from crossing animals of the T×NC variant is more delicate, succulent, and has a total higher quality and consumer properties.

DISCUSSION

The evaluation of “marbling” of muscular tissue is known to demonstrate the presence of intramuscular fat favoring muscular bundles to be loosening, thus improving meat structure and increasing consumer properties.

The analysis of experimental data allowed inducing the general regulations: the thinner muscular fibres, the more fat layers in sheep muscular tissue, and therefore the numerical score of marbling is higher.

Marbling has much stronger and more predictable effects on juiciness and flavour than tenderness (NHAT THU, 2006), the direction of Livestock production is to provide meats of high and consistent eating quality (HOCQUETTE *et al.*, 2010). The intramuscular fat (IMF) was found to be a key element of eating quality that interacts both positively and negatively with a range of other factors (HOCQUETTE *et al.*, 2010; JACOB and PETHICK, 2014). The above statements of the authors supported the result of our study.

Thereby, alongside with general regularities of sheep muscular tissue depending on genotype reliable interbred differences in number and diameter of muscular fibres, connective tissue content have been pointed out. However, the standard chemical analysis does not permit to reveal reliable differences.

JOO *et al.* (2013) reviewed that some muscles contain relatively large quantities of connective tissue, which associated with meat toughness, that if meat contains either unexpected excess or low amount of fat, it would be considered low quality meat. Likewise, stated that fat content, connective tissue, and muscle fiber characteristics have a significant influence on meat quality.

LISTRAT *et al.* (2016) also reviewed the biochemical and structural characteristics of muscle fibers, intramuscular connective tissue, and the intramuscular fat appear to play independent role, suggested that properties of those various muscle components could independently modulate by the genetics and or environmental factors to achieve production efficiency and improve quality of meat. Other authors reported that typically, the selected breeds for meat production have a greater number of muscle fibers and a smaller amount of intramuscular fat per unit area of muscle (BUNGER *et al.*, 2009; HOPKINS *et al.*, 2011; DE LIMA *et al.*, 2016). MCPHEE *et al.* (2008) found that the amount of intramuscular fat significantly increased as the sheep became older and fatter; however, these differences were quantitatively small. Faster growth in lambs has been shown to result in thinner connective tissue, but there were no interactions with genotype or sire (ALLINGHAM *et al.*, 2009; WARNER *et al.*, 2011). Considerable with ours that by AKSOY *et al.* (2019) found that the dry matter, ash, protein, and intramuscular fat values of LD muscles differed between breeds.

We do agree with PEINADO *et al.*, (2004) of the proposed several factors including the genotype, sex and diet possibly manipulate muscle fibre characteristics and the meat quality in animals. Supported our result as well the comment of other authors of such as ORAMARI *et al.*, (2014) that fat is the most variable tissue in the carcass and it varies not only in its total amount but also its distribution between the various deposits which alter markedly during growth, and

the proportions and location of the fat in the body are important in the meat animals. The variation in the quantity of fat lay down in different parts of the body as well as the percentage contribution is differing, between breeds. TELLAM *et al.*, (2012) opinion that the immediate future of the sheep meat industry will see the increasing exploitation of natural genetic variation contributing to muscling that the enhanced muscling is often associated with the morphology change of animal. Genetic strategies to improve the profitability of sheep operations have generally focused on traits for reproduction. However, natural mutations exist in sheep that affect muscle growth and development, and the exploitation of these mutations in breeding strategies has the potential to significantly improve lamb-meat quality (COCKET *et al.*, 2005). HOPKINS *et al.* (2011) reviewed the influence of sheep genotype on meat quality traits where genotype includes breeds, cross-breeds, and major gene effects. The muscle fibres differed among class and genotype and had an effect on meat quality (CHULAYO and MUCHENJE, 2016).

It can be related and connected to the present study the notes of the authors below. The essence of Animal breeding dealt with the variable, primarily genetic, above all, the genetic improvement of populations (PETROVIC *et al.*, 2017a). The achievements of genetics, valuable genotypes with desired properties provide a choice of starting material for breeding. Likewise, the widespread use of methods of biotechnology and tissue culture cells will significantly speed up the selection process and put it to a qualitatively new basis (PETROVIC *et al.*, 2017b).

CONCLUSION

In terms of the muscular structure, crosses of TxNC showed the best results. The chemical composition of the meat of the observed genotypes is more or less uniform. Based on the results obtained, we can terminate that the genetic influence on the histological structure of muscle tissues has different effects depending on the variants of the crossing. It has shown here that the effect of crossing depends on the selected genotypes to crossed. The genetic distance between NC and T, as well as NC and PD, is evident because of their geographical distance as well as their different breed history. However, cross-breeding our results favor the Texel (T) breed for use on sheep farms to increase meat production.

ACKNOWLEDGEMENTS

This paper is the result of cooperation between The Stavropol State Agrarian University, Stavropol, Russia, The All-Russian Scientific Research Institute of Sheep and Goat breeding, Stavropol, Russia and Institute for Animal Husbandry, Belgrade-Zemun, Serbia.

Received, November 02nd, 2019

Accepted September 12nd, 2020

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GENETIČKI UTICAJ NA HISTOLOŠKU STRUKTURU I HEMIJSKI SASTAV MIŠIČNOG TKIVA OVCE

Vladimir Ivanovich TRUKHACHEV^{1*}, Marina Ivanovna SELIONOVA¹, Irina Ivanovna DMITRIK², Milan P. PETROVIC³, Violeta Caro PETROVIC³, Dragana RUZIC-MUSLIC³, Nevena MAKSIMOVIC³

¹Ruski državni poljoprivredni univerzitet – Timirjazev poljoprivredna akademija, Moskva, Rusija

²FGBNU Sveruski istraživački institute za oplemenjivanje ovaca i koza, Stavropolj, Rusija

³Institut za stočarstvo, Beograd, Srbija

Izvod

Proučavan je efekat različitih genotipova ovaca, hranjenih na isti način, na mikrostrukturu i hemijski sastav mišićnog tkiva (*musculus longissimus dorsi*). Cilj studije je da se vidi kako kombinacija gena različitih rasa utiče na pomenuti problem. Otkriveno je da je mišićno tkivo ovaca dobijeno ukrštanjem severno-kavkaskih rasa (NC) i Tekel (T) odlikovano većom količinom mišićnih vlakana za 4,73% i 10,14% ($P < 0,05$), a manjim prečnikom za 5,65 % i 18,6% ($P < 0,05$), većom ocenom mernosti za 5,2 i 8,3 poena, manjim sadržajem vezivnog tkiva za 0,8 i 1,3 apsolutnog procenta u poređenju sa punokrvnim severno-kavkaskim i ukrštanjima severno-kavkasko I Poll Dorset rase (PD). Pokazalo je da je meso dobijeno ukrštanjem životinja T × NC varijante sočnije i ima ukupno viši kvalitet za potrošačke potrebe. Rezultati različitih ukrštanja ukazuju na opravdanost upotrebe morfoloških karakteristika mišićnog tkiva na histološkom nivou kada se ocenjuje kvalitet pre klanja ovaca.

Primljeno 02. XI.2019

Odobreno 12. IX. 2020.