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The Effect of Point Mutations in the RYR-1 Gene on the Physicochemical Properties of Meat

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Abstract

The impact of gene mutations in the RYR-1 gene on the physicochemical qualities and nutritional value of homozygous and heterozygous stress-resistant NOR carcasses with RSE and DFD defects was studied using molecular genetics methods. NOR, which is meat of homozygous cross-breeding pigs, had a 0.44 higher pH level, 2.62% higher water-holding ability, 2.36 units×10^3 higher color and 0.37% higher content of organic matter; the tryptophan content and protein-quality index were lower by 0.3 mmol/g and 0.29, respectively. This low acidity caused the denaturation of some sarcoplasmic proteins, which contributed to the loss of the meat's water-holding ability. A sharp decrease in pH led to partial denaturation of the sarcoplasmic proteins, which determined the pale color of the PSE meat. It was found that, compared to the heterozygous stress-sensitive animals with DFD defects, the NOR pH level was 0.55 lower, the water-holding ability was 4.05% lower, and the color intensity was 4.97 units×10^3 lower; the protein-quality indicator amounted to 8.24, which was 1.12 higher than that in the heterozygous stress-sensitive animals with DFD defects. The defects had a significant impact on other physicochemical properties of the meat. Defects of PSE meat worsened meat color. The intensity of staining of muscle tissue with PSE defects was lower than in normal pork by 11.5, 8.7 and 6.4 units×10^3, respectively. DFD-pork exceeded normal meat in color by 13.7 (P> 0.99), 9.5 (P> 0.99) and 7.4 units×10^3.

Keywords: stress tolerance, stress sensitivity, genotype, meat defects PSE and DFD-, RYR-1 gene, pH, water-holding ability, meat coloring.

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1. Introduction

Studies of the defects of PSE and DFD confirmed that one of the causes of defects is a mutation in the ryanodine receptor gene (RYR -1), which encodes the synthesis of one of the muscle cell proteins [1, 2].

Sensitivity to stress, malignant hyperthermia (MH) depends on the genotype of the RYR-1 ryanodine receptor gene, with the dominant N allele characterizing resistance, and the recessive allele n characterizing stress. Regardless of the partner's genotype, all offsprings with the NN genotype are stress resistant. Sensitivity of the offspring inherited from the hidden Nn carrier depends on the genotype of the second parent [3, 4].

A mutation in the RYR-1 gene is the cause of the incorrect structure of the ryanodine receptor protein, associated with a change in the concentration of calcium ions.

A defect in the structure of this protein leads to the fact that the normal flow of calcium ions into the cytoplasm is impossible. P. J. O. Brien found that the concentration of ionized calcium in the muscle cells of animals with a MH syndrome is 4.6 times lower than in the muscle cells of healthy animals [5].

The research aim is to determine the physicochemical and chemical properties of meat with PSE and DFD signs.

2. Methods

DNA genotyping of pigs was carried out using polymerase chain reactions according to the Mullis' method, improved in [6] and modified in [7].

Animals were slaughtered out according to generally accepted methods in slaughter shops of farms and OJSC "Medvedovsky Meat Processing Plant" in the Timashevsky District of Krasnodar Territory. After 24-hour cooling at + 2... + 40 ° C, slaughter mass, fat thickness, mass of the back third of the carcass, and the "muscle eye" were analyzed. To study the physicochemical properties and chemical composition of meat, samples of the longest back muscle (400 g) were taken in the area between 9-12 thoracic vertebrae.

A chemical analysis of muscle and adipose tissue was carried out according to the generally accepted method of zooanalysis [8].

Amine nitrogen was determined by the method of formol titration, lactic acid by the Friedemann's method [9].

The content of oxyproline was determined by the method of Neumann-Logan modified by Verbitsky and Detereydzh, the content of tryptophan was determined by the method developed by S. E. Gyrehem, E.P.Smith, S.W. Hier, D.L. Klein using an alkaline hydrolysis according to E. Werbicki, F.E. Deatherage.

The main meat defects were PSE (pale, soft, exudative meat) and DFD (dark, fat, dry meat).

According to the standards developed by VNIIMP, meat whose pH is below 5.80 refers to PSE; normal meat has pH of 5.81-6.20, and DFD has pH which is above 6.21.

3. Results

We found that in homozygous and heterozygous stress-resistant gilts half meat is crude (1/2 CM-1) + half - Lanras (1/2 L) meat. 24 hours after slaughter pH was normal, and in 6 heterozygous stress sensitive animals, 4 carcasses had PSE defects and three carcasses had DFD defects.

An analysis of the data obtained (Table 1) indicates that meat with the PSE signs had a lower water-holding ability (by 6.67%), and a tendency to worse values in color, content of “raw fat; at the same time, it had more “raw” ash and tryptophan, and a higher protein-quality indicator (by 1.41).

Table 1: Physical and chemical properties and chemical composition of meat of experimental animals

Indicator	NN n= (31)	Nn (n=16)	PSE (n=4)	DFD (n=3)
pH	5.82±0.06	5.91 ± 0.01	5.38±0.03**	6.37±0.1**
Water holding capacity, %	71.26±3.15	73.28 ±2.96*	68.64±1.62**	75.31±1.84**
Intensity of coloring, units ext.×10^3	66.34±3.67	66.89± 3.21	63.98±2.31**	71.11±2.36**
Water, %	74.14±0.23	73.46±0.18*	74.48±0.47*	74.19±0.36
Dry matter, %	25.86±0.08	26.54±0.05*	25.52±0.11	25.81±0.12
Crude ash, %	1.12±0.02	1.14±0.01	1.15±0.02	1.13±0.03
Organic matter, %	24.74±0.08	25.40±0.04	24.37±0.14	24.68±0.11
Crude protein, %	22.26±0.07	22.72±0.02	22.09±0.09*	22.20±0.10
Crude Fat, %	2,48±0.11	2.68±0.09	2.28±0.10*	2.48±0.13
Tryptophan, mmol / g	18.13±0.09	18.81±0.04*	18.43±0.12	18.08±0.14
Oxyproline, mmol / g	2.20±0.04	2.32±0.07*	2.16±0.06	2.54±0.08*
Protein-quality indicator	8.24	8.11	8.53	7.12

Note: P>0.95*; P>0.99**; P>0.999***

When considering the physicochemical properties and chemical composition of NOR with PSE defects, it was identified that the pH, water-holding ability, color of meat, and the content of organic matter were 0.44, 2.62% (P > 0.99), 2.36 units×10^3 (P> 0.95) 0.37% higher, and the tryptophan content and protein-quality index were 0.3 mmol/g and 0.29 lower, respectively [10, 11].

This acidity is the cause of denaturation of sarcoplasmic proteins, which contributes to the loss of the water-holding ability of meat [12, 13]. Water content is determined

by the rupture of cell membranes, as a result of which the cell fluid quickly fills the intercellular space.

A sharp decrease in pH leads to partial denaturation of sarcoplasmic proteins, which determines the pale color of PSE meat. Pork becomes pale. A similar phenomenon of reducing the quality of pork was called the syndrome of fawn, soft, exudative state of the muscles - PSE (pale, soft, exudative). Products made from PSE meat become sour during storage [14].

Our data are consistent with the results of other researchers [15]. When considering NOR according to their physicochemical properties and chemical composition, it was found that the pH level, values of the water-holding ability, and color intensity were lower by 0.55 (P> 0.95), 4.05% (P> 0.99), and 4.97 units× 10^3(P> 0.99), respectively, and the protein-quality indicator amounted to 8.24, which is 1.12 higher than in heterozygous stress-sensitive animals with DFD-meat defects.

Throughout the post-slaughter period, the pH level was higher in the samples of DFD-pork. 45 minutes, 1 and 2 days after, the pH value in pork with DFD defects was higher than in normal meat by 0.51 (P> 0.999), 0.35 (P> 0.999), 0.31 (P> 0.999). In PSE pork, the pH level of muscle tissue was lower than in NOR meat by 0.20 (P> 0.95), 0.26 (P> 0.99), 0.22 (P> 0.99), respectively.

The pH value of meat corresponded to the categories of NOR-, PSE- and DFD-pork. For all muscle tissue samples, a daily decrease in the pH level was observed, the most significant values were 5.71-4.82 on the sixth day. For DFD-meat, a sharp decrease in the pH level was observed during the first 24 hours. Then the process of reduction was uniform. For NOR-pork, the daily decrease in meat pH was uniform and amounted to 0.07–0.13 [16].

Similar trends were observed for the dynamics of the value of the water-holding ability.

Meat with PSE defects was characterized by lower HCL values in the post-slaughter period; on the first and second days after slaughter, the indicator was 12.3 (P> 0.95), 13.3 (P> 0.95), 11.2 (P> 0.95) lower than that in NOR pork. Pork with a DFD defect was characterized by values of the water holding ability which were higher by 8.6 (P> 0.95), 5.0 (P> 0.95), 7.4 (P> 0.95), respectively.

The VUS value for all pork categories corresponded to the established criteria. In contrast to the pH level, the value of the water-holding ability had its own characteristics. As for the pH level, the WCS value had maximum values for fresh meat (45 minutes after slaughter). For NOR and DFD meat, the level of WCS was decreasing during 7 hours (for PCE pork - during 96 hours) after slaughter with a subsequent increase in the

values of the WCS. However, the WCS values for all categories did not reach the level of the fresh meat stage [17].

Defects in meat quality have a significant impact on other physicochemical properties of meat. Defects of PSE meat significantly reduce the color of meat. The intensity of staining of muscle tissue with PSE defect was lower than that in normal pork by 11.5 (P> 0.99), 8.7 (P> 0.99) and 6.4 units×10^3 (P> 0.99), respectively. DFD-pork had color values higher by 13.7 (P> 0.99), 9.5 (P> 0.99) and 7.4 units×10^3 (P> 0.99) [18].

In all the groups, the brightest muscle tissue was observed in all the categories of fresh meat (1 hour after slaughter). For NOR-meat, an average daily decrease in color was 7.4 units×10^3, for PSE-4.6-5.1 units×10^3, for DFD meat - 9.5-11.3 units×10^3.

During cooking of normal pork, loss of meat juice was 34.1%; in DFD-pork these losses were slightly lower (by 0.3%). Such a negative defect as PSE increases losses during cooking (by 2.6%, P> 0.95).

4. Discussion

Using DNA genotyping for the RYR-1 gene, the selective assessment of stress resistance is more effective. By determining the animal genotype by the RYR-1 gene, it is possible to create a homozygous NN stress-resistant population and get rid of the PSE- and DFD-defects.

5. Conclusion

An increase in the production of high-quality competitive and environmentally friendly livestock products is important for ensuring food security of the Russian Federation. The increased interest in improving productive qualities of pigs had a significant impact on the molecular genetic research.

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References

[1] Grishkova, A. P. and Barkov, D. A. (2015). Use of DNA Markers in Pig Selection of The Factory Type KM-1. *International Journal of Applied and Basic Research*, vol. 3-2, pp. 241-244.

[2] Zinoviev, N. A., *et al*. (2005). Assessment of Animals by Genetic Markers. *Journal of Industrial and Pedigree Pig Breeding*, vol. 3, pp. 18-20.

[3] Ernst, L. K. and Zinoviyeva, N. A. (2008). *Biological Problems of Livestock Breeding in XXI Century*. Moscow: RAAS.

[4] Kurak, O. P., *et al*. (2006). The Influence of the RYR1 Gene on the Quality of Pig Meat. *Journal of Zootechnical Science of Belarus*, vol. 41, pp. 42-48.

[5] Kovalyuk, N. V. (2002). The Use of Genetic Markers of Stress Resistance and Multiple Fertility in Pig Selection. (PhD dissertation, University of Krasnodar, 2002).

[6] Loban, N. A. (2010). Production Level and New Methods of Improving Belorussian Large White Pigs. In *Modern Problems of Intensification of Pork Production in CIS Countries: Collection of Scientific Papers of 17th International Scientific and Production Conference on Swine Breeding Dedicated to the 75th Anniversary of Honored Scientist of the Russian Federation, Professor VE Ulitko.* Ulyanovsk: Breeding, Selection, Genetics and Reproduction of Pigs.

[7] Maksimov, G., Maksimov, A. and Lenkova, N. (2015). DNA-genotyping on RYR-I, ESR and H-FABP alleles and Meat Production of Pigs. *Journal of Chief Zoo Engineer,* vol. 1, pp. 24-31.

[8] Pestis, V. K., *et al*. (2015). Association of Polymorphism of the RYR1 Gene with Indicators of Productive Qualities of Pig Breeds Bred in Belarus. *Journal of Young Scientist*, vol. 5, pp. 33-37.

[9] Polozyuk, O. N. and Maksimov, G. V. (2010). The Effect of Mutations in the Ryr-1 Gene on Biochemical Blood Parameters and Meat Quality of Pigs. *Journal of Pig Production*, vol. 8, pp. 30-31.

[10] Polozyuk, O. N., *et al*. (2014). Gene Markers Influencing Production of Pigs. *Journal of Swine Breeding*, vol. 4. pp. 35-36.

[11] Ryzhova, N. V. and Kalashnikov, L. A. (2002). Productive Qualities of Heterozygous Pigs - Carriers of the Mutant Allele Gene. *Journal of Vestnik of the Russian Agricultural Science*, vol. 3, pp. 64-67.

[12] Sayenko, A. M. and Balatski, V. N. (2009). Polymorphism of RYRI, ESR, PRLR and GH in Pig Populations of Various Breeds. In *Materials of the 18th Meeting of the Interuniversity Coordination Council on Swine Breeding and International Scientific*

and Production Conference "Current problems of pork production in the Russian Federation". Persianovsky: DSAU, vol. 1, pp. 74-77.

[13] Semyonov, V. V., *et al*. (2013). Using RYR-1, ESR and H-FABP Gene Polymorphism on Swine Breeding. *Journal of Herald of the Russia Academy of Agrarian Sciences*, vol. 2, pp. 65-67.

[14] Tarichenko, A. I., Lodyanov, V. V. and Kozlikin, A. V. (2011). Biological and nutritional value of muscle tissue depending on quality defects. In *Materials of the International Scientific and Practical Conference: Innovations in science, education and business - the basis for effective development of the agro-industrial complex*. Persianovsky: DSAU, vol. 3, pp. 291-292.

[15] Shkalenko, V. V., *et al*. (2011). Biological and Nutritional Value of Meat of Gilts of Different Breeds. *Journal of Pig Production*, vol. 4, pp. 32-33.

[16] Getmantseva, L. V., *et al*. (2015). Effect of POU1F1 Gene Polymorphism on Growthand Meat Traits of Pigs. *Journal of Modern Problems of Science and Education*, vol. 2, pp. 746.

[17] Karagodina, N., *et al*. (2014). Influence of Various Bio-Stimulants on the Biochemical and Hematological Parameters in Porcine Blood Plasma. *World Applied Sciences Journal*, vol. 6, pp. 723-726.

[18] Mikhailov, N. V., *et al*. (2013). Promising Genes Markers of Pig Productivity. *Journal of Bulletin of the Don State Agrarian University*, vol. 3, pp. 16-19.