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The effect of genotypes at *loci CAST/Msp*I (calpastatin) and *MYOG* (myogenin) and their interaction on selected productive traits of porkers free of gene *RYR1*^{T}. II. Meat quality.

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The study aimed at determining whether the quality traits of pork are related to the genotype at *loci CAST/Msp*I and *MYOG* and whether an interaction exists between them, affecting the pork quality traits. The analyses were conducted on 397 porkers free of gene *RYR1*^{T} of the five following purebred and crossbred groups: Landrace, Landrace × Yorkshire, Landrace × Duroc, (Landrace × Yorkshire) × Duroc, and (Landrace × Yorkshire) × (Duroc × Pietrain) – 91, 65, 129, 83 and 29 animals, respectively. Among the quality and basic chemical composition traits of pork, the pH value, measured from 24 to 144 h *post-mortem*, depended significantly on the *CAST/Msp*I genotype, while the electric conductivity in meat, measured from 35 min to 24 h *post-mortem*, depended on the genotype *MYOG*. No significant effect of *CAST/Msp*I × *MYOG* interaction was found on meat quality traits and its basic chemical composition.

KEY WORDS: calpastatin / meat quality / myogenin / pig / porkers

Genes *RYR1* and *RN* are called the major genes of carcass and meat quality traits in pigs [Sellier and Monin 1994]. Their effect on carcass and pork quality indicators was confirmed by numerous studies conducted on different purebred and crossbred pigs [Przybylski *et al*. 1996]. Despite this, however, among animals of the same breed

and genotype at *locus RYR1* one may observe significant differences as regards certain indicators of both carcass and meat quality. It is, therefore, necessary to identify other genes determining the phenotypic differentiation of animals in relation to these traits [Koćwin-Podsiadła *et al.* 2006]. The understanding of metabolic routes related to the development and physiology of the muscle and fat tissues, as well as of the metabolic processes taking place in the muscle *post-mortem*, may indicate that one should examine genes in relation to their effect on those traits. Among the genes engaged in the development and metabolism of the muscle tissue are those of calpastatins (*CAST*) and myogenins (*MYOG*).

Calpain-calpastatin system plays an important role in the *post-mortem* protein proteolysis, thus deciding about many meat quality traits, among others its tenderness, water binding capacity and drip loss [Melody *et al*. 2004].

The studies presented here aimed at evaluating the effect of the genotype at *loci MYOG* and *CAST* on meat quality properties, as well as estimating the effect of interaction between them on the traits examined in porkers free of gene $RYR1^T$.

Material and methods

Animals

The investigations covered 397 porkers, including purebred Landrace and the following crossbred pigs: Landrace × Yorkshire, Landrace × Duroc, (Landrace × Yorkshire) × Duroc, and (Landrace × Yorkshire) × (Duroc × Pietrain) – 91, 65, 129, 83 and 29 animals, respectively, all free of gene $RYR1^T$.

Relations examined

Quality of fresh and cooled meat was evaluated post-slaughter of the *longissimus dorsi* (LD) muscle on the basis of the following parametres:

- pH measured directly in the LD muscle 35 min, 2 h, 3 h, 24 h, 48 h, 96 h and 144 h *post-mortem* with a MASTER pH-meter (DRAMINSKI) and 45 min *post-mortem* in a water homogenate of the LD tissue, according to the Polish Standard PH-77/A-82058, using a CP-311 pH-meter (ELMETRON) with a combined glass electrode type OSH-10-OO;
- electric conductivity (EC) measured with a LF-Star conductometer (MATTHAÜS) 35 min, 2 h, 3 h and 24 h *post-mortem*;
- colour brightness (L*) of the LD muscle tissue, measured with MINOLTA CR310 apparatus, 24 h *post-mortem*;
- rate of ATP breakdown, expressed by indicator R1 = IMP/ATP, determined 45 min *post-mortem* according to Honikel and Fischer [1977];
- water holding capacity (WHC), determined 24 h *post-mortem* with the filter paper method according to Grau and Hamm [1952] as modified by Pohja and Ninivaara [1957];

– drip loss, determined according to Prange *et al*. [1977], 48 h, 96 h and 144 h *post- mortem*;
– meat yield in the process of curing and cooking (72°C), expressed by indicator TY, according to Naveau *et al*. [1985], as modified by Koćwin-Podsiadła *et al.* [2004].

The samples cut from the *longissimus lumborum* (*LL*) muscle 45 min *post- mortem* were analysed for the glycolytic potential and content of glycogen and lactic acid. The contents of glycogen, glucose and glucose-6-phosphate were determined with the enzymatic method according to Dalrymple and Hamm [1973], while the level of lactic acid according to Bergmeyer [1974].

The glycolytic potential of the muscle, measured in micromoles of lactic acid per 1 g of tissue, was calculated according to the formula recommended by Monin and Sellier [1985].

Moreover, samples obtained from the LD muscle were analysed for the basic components, *i.e*. water and dry matter contents according to Polish Standard PN-73/A82110, total protein (after Kjeldahl) according to PN-75/A04018 and intramuscular fat (after Soxhlet) according to Polish Standard PN-73/A82111.

The genomic DNA was isolated from blood leukocytes according to Kawasaki [1990]. Genotypes *CAST/Msp*I and *MYOG* were identified with the PCR/RFLP method according to Ernst *et al*. [1998] and Soumillion *et al*. [1997], respectively.

The *AA* genotype at *MYOG locus* was not found in analysed pigs. The remaining genotypes at that *locus* (*AB*, *BB*) and all three genotypes at *CAST*/MspI *locus* (*AA*, *AB*, *BB*) were present in all the genetic groups analysed.

Statistical

The effect of genotypes *CAST* and *MYOG* on the basic composition and quality properties of meat was determined using a two-factor analysis of variance in an non-orthogonal arrangement. The statistical model comprised: *CAST* and *MYOG* genes polymorphism and their interactions:

$$Y_{ijk} = \mu + a_i + b_j + ab_{ij} + e_{ijk}$$

where:

Y_{ijkl} – the meat quality trait;
μ – the overall mean;
a_i – the effect of *CAST* genotype, $i = 1, 2, 3$;
b_j – the effect of *MYOG* genotype, $j = 1, 2$;
ab_{ij} – the effect of the interaction between *CAST* and *MYOG* genotypes;
e_{ijk} – the random error.

Significance of differences between means was identified with the NIR test [STATISTICA 1997, PL 5.1].

Table 1. The effect of *CAST/Msp*I and *MYOG* genes and their interaction on meat quality traits

Trait	*CAST/Msp*I				*MYOG*			Interaction – $F_{emp.}$
	AA (n=100)	*AB* (n=163)	*BB* (n=134)	$F_{emp.}$	*AB* (n=141)	*BB* (n=256)	$F_{emp.}$	
pH_{35}LD	6.59 ±0.18	6.60 ±0.16	6.60 ±0.18	0.12 ns	6.60 ±0.18	6.60 ±0.16	0.17 ns	0.12 ns
pH_{45}LD	6.36 ±0.25	6.37 ±0.28	6.32 ±0.30	1.22 ns	6.34 ±0.27	6.35 ±0.29	0.24 ns	0.08 ns
pH_2 LD	6.38 ±0.23	6.39 ±0.27	6.38 ±0.23	0.04 ns	6.40 ±0.20	6.37 ±0.27	1.35 ns	0.23 ns
pH_3 LD	6.20 ±0.23	6.25 ±0.22	6.21 ±0.22	0.59 ns	6.25 ±0.19	6.21 ±0.23	2.14 ns	1.11 ns
pH_{24} LD	5.63^{B} ±0.10	5.59^{A} ±0.13	5.58^{A} ±0.11	**6.21** **	5.60 ±0.11	5.60 ±0.12	0.21 ns	0.18 ns
pH_{48} LD	5.47^{b} ±0.12	5.44^{ab} ±0.11	5.42^{a} ±0.12	**4.24** *	5.46^{b} ±0.10	5.43^{a} ±0.12	**5.68** *	0.60 ns
pH_{96} LD	5.46^{B} ±0.12	5.42^{A} ±0.12	5.39^{A} ±0.08	**9.01** **	5.43 ±0.12	5.42 ±0.11	0.70 ns	0.20 ns
pH_{144} LD	5.53^{B} ±0.13	5.49^{AB} ±0.13	5.47^{A} ±0.11	**4.97** **	5.50 ±0.13	5.48 ±0.12	2.54 ns	2.29 ns
R1	0.90 ±0.05	0.89 ±0.05	0.89 ±0.03	2.61 ns	0.90 ±0.05	0.89 ±0.04	1.87 ns	1.74 ns
EC_{35} (mS/cm)	3.45 ±0.83	3.53 ±0.68	4.65 ±0.89	2.75 ns	3.79^{B} ±1.01	3.38^{A} ±0.55	**15.15** **	2.32 ns
EC_2 (mS/cm)	3.18 ±0.94	3.06 ±0.76	3.18 ±0.89	1.45 ns	3.07 ±0.88	3.16 ±0.83	0.69 ns	0.91 ns
EC_3 (mS/cm)	3.27 ±1.05	3.20 ±0.86	3.07 ±0.84	1.42 ns	3.39^{B} ±1.06	3.04^{A} ±0.80	**12.33** **	1.54 ns
EC_{24} (mS/cm)	3.89 ±1.31	3.68 ±1.14	3.88 ±1.17	1.47 ns	4.10^{B} ±1.27	3.63^{A} ±1.12	**14.95** **	0.49 ns
Meat brightness (L*) of LD	54.07 ±2.89	54.31 ±3.16	54.39 ±2.88	0.48 ns	54.63 ±2.98	54.08 ±2.99	2.64 ns	0.26 ns

Table 1. Continued.

Trait	*CAST/Msp*I				*MYOG*			Interaction – $F_{emp.}$
	AA (n=100)	*AB* (n=163)	*BB* (n=134)	$F_{emp.}$	*AB* (n=141)	*BB* (n=256)	$F_{emp.}$	
Drip loss at 48h (%)	6.24 ±2.82	6.49 ±2.53	6.74 ±2.51	0.84 ns	6.42 ±2.41	6.56 ±2.70	0.39 ns	1.22 ns
Drip loss at 96h (%)	9.76 ±3.54	9.87 ±3.09	10.71 ±3.02	1.82 ns	9.83 ±2.72	10.28 ±3.42	1.66 ns	1.22 ns
Drip loss at 144h (%)	12.18 ±3.54	12.23 ±3.09	12.98 ±3.22	1.59 ns	12.69 ±3.24	12.33 ±3.26	1.19 ns	1.18 ns
WHC (cm^2)	5.27 ±1.35	5.35 ±1.58	5.28 ±1.54	0.41 ns	5.47 ±1.36	5.21 ±1.58	1.64 ns	2.49 ns
TY (%)	103.63 ±3.83	103.72 ±4.62	102.75 ±4.86	2.40 ns	103.11 ±4.60	103.52 ±4.50	0.38 ns	1.10 ns
Glycolytic potential (µmol/g)	122.35 ±26.30	126.82 ±21.75	128.33 ±28.47	1.41 ns	126.54 ±24.15	126.63 ±26.15	0.81 ns	1.21 ns
Glycogen (µmol/g)	43.57 ±15.64	46.52 ±12.84	47.38 ±14.22	1.52 ns	46.72 ±13.67	46.08 ±14.11	0.39 ns	1.14 ns
Lactate (µmol/g)	35.19 ±10.07	34.47 ±9.90	33.56 ±10.85	0.18 ns	33.09 ±9.62	34.87 ±10.61	0.47 ns	0.16 ns
Water content (%)	75.0 ±0.64	74.84 ±0.79	74.80 ±0.70	1.42 ns	74.85 ±0.72	74.87 ±0.73	0.06 ns	0.07 ns
Dry matter content (%)	23.85 ±1.06	23.70 ±1.01	23.76 ±0.94	0.43 ns	24.03^{B} ±1.07	23.60^{A} ±0.91	**11.45** **	0.42 ns
Total protein content (%)	22.29^{a} ±0.62	22.36^{ab} ±0.58	22.53^{b} ±0.61	**3.26** *	22.51 ±0.63	22.34 ±0.58	3.72 ns	0.08 ns
Intramuscular fat content (%)	1.72 ±0.62	1.70 ±0.70	1.71 ±0.65	0.12 ns	1.71 ±0.60	1.70 ±0.70	0.15 ns	1.43 ns

$^{aA...}$Means within rows bearing different superscripts differ significantly at: small letters – P≤0.05; capitals – P≤0.01.
***P≤0.05; **P≤0.01**.

Results and discussion

Among the 26 traits of meat quality and meat chemical composition, the pH measured directly in the LD muscle during its maturation and storage (from 24 to 144 h *post-mortem*) as well as crude protein content, were found significantly related to genotype *CAST/Msp*I (Tab. 1). The lowest, statistically confirmed pH values and simultaneously the highest crude protein content of the LD muscle were recorded for animals with genotype *BB*.

It is known, that the activity of calpastatin as a calpain inhibitor decreases with decreasing pH, determining not only the rate, but also the range of proteolysis [Geesing and Koohmaraie 1999]. The results of the present study indicate that the selection of animals for a specific *CAST/Msp*I genotype could significantly affect the *post-mortem* pH of meat, *i.e*. alter one of its most important qualitative and technological parametres.

The *MYOG* genotype had a significant effect on the LD pH measured 48 h *post-mortem* ($P \leq 0.05$), its electric conductivity 35 min, 3 h and 24 h *post-mortem* and dry matter content ($P \leq 0.01$). No significant interaction *MYOG* × *CAST/Msp*I in meat quality traits measured was identified

It has not been earlier indicated in literature that the porkers' *MYOG* genotype affected significantly the parametres of meat quality. The highly significant relation between electric conductivity and the *MYOG* genotype observed in this study, shows how important the *MYOG* genotype may be for this trait. However, it is hard to conclude about the mechanism of this relation as the current knowledge about the role of myogenin is limited exclusively to the processes of myogenesis.

Summarizing, it can be stated that among the traits of quality and chemical composition of pork, the pH measured over the period lasting from 24 to 144 h *post-mortem* proved significantly related to genotype *CAST/Msp*I, while electric conductivity measured from 35 min to 24 h *post mortem*, was found to be related to the *MYOG* genotype.

No significant effect of interaction between genotypes *CAST/Msp*I and *MYOG* in relation to meat quality traits and its chemical composition was identified.

REFERENCES

1. BERGMEYER H.U., 1974 – Methods of enzymatic analysis. Academic Press, New York.
2. DALRYMPLE R.H., HAMM R., 1973 – A method for extracting of glycogen and metabolites from a single muscle sample. ***Journal of Food Technology*** 8, 439-444.
3. ERNST C.W., ROBIC A., YERLE M., WANG L., ROTHSCHILD M.F., 1998 – Mapping of calpastatin and three microsatellites to porcine chromosome 2q2.1-q2.4. ***Animal Genetics*** 29, 212-215.
4. GEESINK G.H., KOOHMARAIE M., 1999 – Effect of calpastatin on degradation of myofibrillar proteins by μ-calpain under postmortem conditions. ***Journal of Animal Science*** 77, 2685-2692.
5. GRAU R., HAMM R., 1952 – Eine einfache Methode zur Bestimmung der Wasserbindung im Fleisch. ***Fleischwirtschaft*** 4, 295-297.

6. HONIKEL K.O., FISCHER H., 1977 – A rapid method for the detection of PSE and DFD porcine muscles. ***Journal of Food Science*** 42,1633-1636.
7. KAWASAKI E.S., 1990 – Sample preparation from blood, cells and other fluids. In: PCR Protocols: A guide to methods and applications (M.A. Innis, D.H. Gefland, J.J. Sninsky, T.J. White, eds). Academic Press, New York, pp. 3-12.
8. KOĆWIN-PODSIADŁA M., KRZĘCIO E., ZYBERT A., ANTOSIK K., SIECZKOWSKA H., KURYL J., 2006 – The interactive effects of RN^- gene and calpastatin (CAST) gene for meat quality traits. ***Animal Science*** 1, Supplement, 198-199.
9. KOĆWIN-PODSIADŁA, M., KURYŁ, J., KRZĘCIO, E., ANTOSIK, K., ZYBERT, A., SIECZKOWSKA, H., 2004 – An association between genotype at the CAST (calpastatin) *locus* and carcass quality traits in porkers free of $RYR1^T$ allele. ***Animal Science Papers and Reports*** *22*, 497-505.
10. MELODY J.L., LONERGAN S.M., ROWE J., HUIATT T.W., MAYES M.S., HUFF-LONERGAN E., 2004 – Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. ***Journal of Animal Science*** 82, 1195-1205.
11. MONIN G., SELLIER P., 1985 – Pork of low technological quality with a normal rate of muscle pH fall in the immediate post mortem period: the case of the Hampshire breed. ***Meat Science*** 13, 49-63.
12. NAVEAU J., POMMERET P., LECHAUX P., 1985 – Proposition d'une méthode de mesure du rendement technologique: la "méthode Napole". ***Techni-Porc*** 8, 7-13.
13. POHJA N.S., NINIVAARA F.P., 1957 – Die Bestimmung der Wasserbindung des Fleisches mittels der Konstandrückmethods. ***Fleischwirtschaft*** 9, 193-195.
14. PRANGE H., JUGERT L., SCHARNER E., 1977 – Untersuchungen zur Muskelfleischqualität beim Schwein. ***Archiv für Experimentelle Veterinärmedizin***. Leipzig. 31, 235-248.
15. PRZYBYLSKI W., KOĆWIN-PODSIADŁA M., KACZOREK S., KRZĘCIO E., NOVEAU J., MONIN G., 1996 – Efekt genu RN^- w zakresie cech jakości mięsa świń pochodzących z krzyżowania rasy wbp, linii pbz-23 oraz knurów linii P-76 i rasy hampshire. ***Zeszyty Naukowe Przeglądu Hodowlanego*** 26, 143-149.
16. SELLIER P., MONIN G., 1994 – Genetics of pig meat quality: a review. ***Journal of Muscle Foods*** 5, 187-219.
17. SOUMILLION A., ERKENS J.H.F., LENSTRA J.A., RETTENBERGER G., TE PAS M.F.W., 1997 – Genetic variation in the porcine *myogenin* gene locus**.** ***Mammalian Genome*** 8, 564-568.
18. STATISTICA – 1997, PL.5.1.

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Cechy produkcyjne tuczników wolnych od genu *RYR1^T* oceniane zależnie od genotypu względem *loci CAST*/*Msp*I (kalpastatyny) i *MYOG* (miogeniny). II. Cechy jakości i podstawowego składu mięsa

S t r e s z c z e n i e

Celem badań było określenie, czy cechy jakości mięsa świń są istotnie zależne od genotypu względem *loci CAST*/*Msp*I i *MYOG* oraz czy istnieje współdziałanie między tymi *loci* w zakresie kształtowania się badanych cech. Badania przeprowadzono na 397 tucznikach wolnych od genu *RYR1^T*, następujących ras i ich mieszańców: landrace, landrace × yorkshire, landrace × duroc, (landrace × yorkshire) × duroc i (landrace × yorkshire) × (duroc × pietrain) – odpowiednio 91, 65, 129, 83 i 29 zwierząt. Spośród analizowanych cech jakości i składu podstawowego mięsa, wartości pH mierzone między 24 a 144 godziną *post-mortem* były istotnie zależne od genotypu *CAST*/*Msp*I. Przewodność elektryczna mięsa mierzona w kilku terminach między 35 minutą a 24 godziną po uboju była uzależniona od genotypu *MYOG*. Nie stwierdzono istotnego wpływu interakcji między genotypami *CAST*/*Msp*I a *MYOG* na cechy jakości mięsa i jego skład podstawowy.