

Relationships between the polymorphism of myosin heavy chains and selected meat quality traits of pigs with different susceptibility to stress

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Abstract

The aim of the investigations was to analyse the share of myosin heavy chains (MHC) isoforms (type I, IIa, IIb, and IIx) in the *longissimus thoracis et lumborum* muscle derived from pigs of different *RYR1* genotypes (TT – homozygous negative, CT – heterozygous, CC – homozygous positive). The composition of the MHC isoforms in the muscle tissue of the examined animals was referred to selected meat quality traits. It was revealed that the animals with the CT and TT genotypes were characterized by a significantly ($P \leq 0.05$) lower share of the type I and higher share of the type IIb MHC isoform in comparison to homozygotes CC. Inferior tenderness and water holding capacity of meat obtained from pigs susceptible to stress (TT) at 144 h after slaughter could have been associated, among others things, with the increased share of MHC isoform type IIb. The composition of MHC isoforms might be a useful indicator in breeding work in the selection of animals carrying the gene of susceptibility to stress.

Keywords: pig, ryanodine receptor, *RYR1* gene, MHC isoforms, meat quality

Zusammenfassung

Zusammenhang zwischen dem Myosin Polymorphismus und ausgewählten qualitativen Fleischmerkmalen bei Schweinen mit unterschiedlicher Stressempfindlichkeit

Analysiert wurden verschiedene Isoformen für die schweren Ketten des Myosins (MHC) der Typen I, IIa, IIb und IIx im Rücken- und Lendenmuskel (*M. longissimus thoracis et lumborum*) von Schweinen verschiedener Ryanodin-Rezeptoren (TT – homozygot negativ, CT – heterozygot, CC – homozygot positiv). Die verschiedenen MHC-Isoformen im Muskelfleisch wurden mit ausgewählten Fleischqualitätsmerkmalen verglichen. Tiere der Genotypen CT und TT waren im Gegensatz zu den homozygoten CC-Typen durch einen geringeren Anteil des Isoform-MHC-Typs I und einen größeren Anteil des Typs IIb charakterisiert. Geringere Zartheit und Wasserbindungsvermögen des Fleisches 144 Stunden nach der Schlachtung könnte bei den stressempfindlicheren TT-Schweine neben anderen Gründen

mit einem höheren Anteil des Isoform-MHC-Typs IIb im Zusammenhang stehen. Die Zusammensetzung der MHC-Isoformen stellt möglicherweise einen nützlichen Indikator für die Zuchtarbeit zur Eliminierung von Genen der Stressanfälligkeit dar.

Schlüsselwörter: Schwein, Ryanodin-Rezeptor, *RYR1*, Myosin schwere Ketten (MHC), MHC-Isoformen

Introduction

Quality of pork depends on many factors, among which a special role is played by genetic factors (SELLIER and MONIN 1994, VON LENDERKEN *et al.* 1994, DE VRIES *et al.* 2000). A major gene determining meat quality and susceptibility of pigs to stress is the ryanodine receptor gene (*RYR1*), previously known as the halothane gene (*HAL*) (FUJII *et al.* 1991, SELLIER and MONIN 1994, LUNDSTRÖM *et al.* 1995, DOVC *et al.* 1996, KOĆWIN-PODSIADŁA *et al.* 2000, KRZĘCIO *et al.* 2004, KUSEC *et al.* 2005, WOJTYSIAK and MIGDAŁ 2007). A mutated allele of *RYR1* is responsible for the occurrence of the PSE defect of meat, especially in pigs with high meat contents in the carcass. An additional adverse effect of these anomalies is the occurrence of malignant hyperthermia (the MH syndrome), resulting in the denaturation of contractile protein (myosin) and sarcoplasmic protein (myoglobin), and thus in the deterioration of many meat quality attributes (BOLES *et al.* 1991, LUNDSTRÖM *et al.* 1995, MARTENS 1998).

The adverse effect of the *RYR1* gene on meat quality, as well as its processability and eating quality, was confirmed in numerous studies (BOLES *et al.* 1991, DE SMET *et al.* 1998, ESSÉN-GUSTAVSSON *et al.* 1992, LUNDSTRÖM *et al.* 1995, GÖDEKE *et al.* 1998, THALLER *et al.* 2000, KRZĘCIO *et al.* 2004). Significant dependencies were found between traits of muscle fibres, susceptibility to stress and meat quality (ESSÉN-GUSTAVSSON *et al.* 1992, FIEDLER *et al.* 1993, 2001, PEDERSEN *et al.* 2001, DEPREUX *et al.* 2002, EGGERT *et al.* 2002, RYU and KIM 2005). The discovery of immunohistochemical methods made it possible to distinguish types of muscle fibres based on specific isoforms of myosin heavy chains (MHC) (SCHIAFFINO and REGGIANI 1996, GREASER *et al.* 2001, PONSUKSILI *et al.* 2008) and relate them to muscle quality.

The aim of the study was to analyze the proportions of isoforms of myosin heavy chains (MHC) in *m. longissimus thoracis et lumborum* collected from pigs with different *RYR1* genotypes. The composition of MHC isoforms in the muscle tissue of tested animals was referred to selected quality attributes of their meat.

Material and methods

The experimental material was *m. longissimus thoracis et lumborum*, which was collected from a total of 66 porcine half-carcasses, including 36 crossbred fatteners (Landrace × Yorkshire) × Duroc [(L×Y)×Dur] and 30 fatteners of Line 890 (Line 990 × Pietrain) with varied susceptibility to stress. The parental generation of (L×Y)×Dur crosses was imported from Denmark. Porkers of Line 890 were obtained from the Experimental Farm, Pawłowice, and were maintained at the Experimental Animal Nutrition Farm, Gorzyń belonged to Poznań University of Life Sciences. Feeding of animals from which muscles

were collected, as well as their management conditions, were controlled. Genotypes of analyzed animals in terms of locus *RYR1* were identified using PCR-RFLP (FUJII *et al.* 1991). A total of 30 animals were diagnosed with genotypes CC, 18 – with genotype CT, and 12 – with genotype TT. Only (L×Y)×Dur crosses were free from the gene of susceptibility to stress.

Pigs were slaughtered under standard abattoir conditions using electrical stunning. Samples (2-5 g) for electrophoretic analyses were collected 45 min after slaughter from the *longissimus* muscle and stored at –80°C until analyzed. The remaining part of the analyzed muscle after cooling was divided into two halves, of which the thoracic section (*thoracis*) was used in analyses after 48 h, while the lumbar section (*lumborum*) 144 h after slaughter. Prepared portions (of 400-600 g) after vacuum packaging were transported to the laboratory under cold-storage conditions in order to assess tenderness and water holding capacity of meat from analyzed animals.

Electrophoretic analyses concerned the determination of isoforms of myosin heavy chains (MHC). Their separation in the fraction of washed myofibrils collected from the muscle tissue 45 min after slaughter was run in 8% PAGE-SDS (MOZDZIAK *et al.* 1998) with an SE 260 apparatus by Hoefer Scientific Instruments. Washed myofibrils were collected as a result of double washing of the muscle tissue with a phosphate buffer solution, the rigor buffer (75 mM KCL, 10 mM KH₂PO₄, 2 mM MgCl₂, 2 mM EGTA with pH 7.0, 0.1 M PMSF) (FRITZ *et al.* 1989). Each sample transferred onto gel contained 0.5 µg protein. Electrophoresis was run at a constant voltage of 70 V at 4°C for 24 h. After the separation was completed gels were stained in a solution containing 0.05% Coomassie blue R-250, 45% methanol and 9.2% acetic acid and next destaining in a mixture of 10% methanol and 7.5% acetic acid. Quantitative analysis of separated bands of MHC isoforms was conducted with an Image Master VDS scanning densitometer by Pharmacia, using Image Master1D Elite version 4.00 software. Computations were based on the assumption that the area of a single protein band accounts for a percentage ratio in relation to the area of all separated proteins in a given sample on gel, which constitutes 100%.

Instrumental assessment of meat tenderness was conducted on the basis of shear force required to cut 10×10 mm samples in an Instron 1140 universal testing machine with a Warner-Bratzler device (WBSF), following thermal processing. Meat slices with a thickness of 25-30 mm were heated in a Rational Combi convection oven in hot air at 160°C for approx. 15 min (GRZEŚ *et al.* 2005).

Sensory examination of meat tenderness on heated meat samples was conducted using the linear scaling method (ADAMIK 1997, BARYŁKO-PIKIELNA 1990). Desirability scores were analyzed in a 0-10 point scale (»0« corresponded to very tough meat, »10« to very tender meat).

Water holding capacity was determined based on centrifugal and thermal drip from the muscle tissue (HONIKEL 1987) as a result of centrifugation (at 25 000× g for 20 min at 20°C) or heating, respectively. The volume of drip, expressed in per cent, was calculated from the difference of meat weights before and after centrifugation or heating.

Results were subjected to a one-way analysis of variance (ANOVA) in a non-orthogonal system due to the unequal number of animals in the treatments using Statistica 8.0 PL software (STANISZ 2000). The significance ($P \leq 0.05$) of differences between the animal groups segregated according to the *RYR1* genotypes for individual meat quality traits was determined using the Fisher test.

Results

Results of the analysis of variance presented in the table showed a statistically significant ($P \leq 0.05$) effect of the gene of susceptibility to stress (*RYR1*) on the composition of muscle fibres corresponding to specific isoforms of myosin heavy chains (MHC), as well as tenderness and water holding capacity of meat in case of analyzed pigs.

Out of the analyzed four types of MHC isoforms (I, IIa, IIb, and IIx) the content of only two of them, i.e. IIb and I, was significantly ($P \leq 0.05$) varied between analyzed *RYR1* genotypes (Table). At the same time it was observed that the proportion of MHC isoform type IIb (corresponding to fast glycolytic fibres) was significantly ($P \leq 0.05$) higher in the groups of CT (68.87%) and TT fatteners (68.87%) in comparison to CC animals (62.76%). In turn, shares of MHC isoform type I (corresponding to slow oxidative fibers) was significantly ($P \leq 0.05$) lower in the group of *RYR1*^T carriers (3.24% for CT, 3.70% for TT) in comparison to animals which were not carriers of this gene (9.33%).

Analysis of meat tenderness both in case of instrumental and sensory examination showed significant ($P \leq 0.05$) dependencies only at 144 h after slaughter (Table). At that time point a significantly ($P \leq 0.05$) lower shear force value, i.e. superior tenderness, was recorded for stress resistant animals (CC) (33.79 N/cm²) in comparison to both CT (47.33 N/cm²) and TT groups (47.61 N/cm²). In turn, in sensory examination of tenderness significant ($P \leq 0.05$) differences were found between TT (5.63 points) vs. CT (6.98 points) and CC genotypes (7.31 points).

Table
Effect of the *RYR1* gene on selected meat quality traits
Einfluss des RYR1 Gens auf ausgewählte Fleischeigenschaften

Selected traits	Genotype <i>RYR1</i>		
	CC, n=36	CT, n=18	TT, n=12
Share of MHC isoforms in the longest muscle, %	27.91 ^a ± 8.98	25.97 ^a ± 6.89	27.75 ^a ± 5.73
Type IIa/IIx	62.76 ^a ± 8.84	70.80 ^b ± 7.39	68.87 ^b ± 6.54
Type IIb	9.33 ^b ± 2.46	3.24 ^a ± 1.40	3.70 ^a ± 1.33
Type I			
Shear force, N/cm ²			
48 h	61.21 ^a ± 20.22	65.33 ^a ± 14.29	65.29 ^a ± 6.69
144 h	33.79 ^a ± 5.48	47.33 ^b ± 15.45	47.61 ^b ± 16.38
Tenderness, scores			
48 h	6.60 ^a ± 1.18	6.59 ^a ± 1.60	6.12 ^a ± 1.21
144 h	7.31 ^b ± 1.06	6.98 ^b ± 1.74	5.63 ^a ± 1.56
Centrifugal drip, %			
48 h	20.22 ^a ± 3.16	20.83 ^{ab} ± 1.98	22.51 ^b ± 3.22
144 h	13.66 ^a ± 4.63	14.82 ^{ab} ± 3.79	17.57 ^b ± 4.74
Thermal drip, %			
48 h	24.75 ^a ± 7.38	29.76 ^b ± 2.34	31.03 ^b ± 2.34
144 h	23.99 ^a ± 8.90	29.56 ^b ± 2.48	30.55 ^b ± 2.71

^{a,b}Mean values from the same row with various letters differ statistically significantly at $P \leq 0.05$.

Analysis of water holding capacity based on the volume of centrifugal and thermal drip showed statistically significant ($P \leq 0.05$) dependencies both at 48 h and 144 h after slaughter (Table). In that case volumes of centrifugal drip were significantly ($P \leq 0.05$) varied between TT (after 48 h – 22.51%, after 144 h – 17.57%) and CC homozygotes (after 48 h – 20.22%, after 144 h – 13.66%). In turn, volumes of thermal drip were significantly ($P \leq 0.05$) varied between CC (after 48 h – 24.75%, after 144 h – 23.99%) and CT (after 48 h – 29.76%, after 144 h – 29.56%) and TT groups (after 48 h – 31.03%, after 144 h – 30.55%). The lowest volumes of centrifugal and thermal drip were recorded for meat sampled from stress resistant animals (Table).

Discussion

In porcine skeletal muscles four types of muscle fibres (I, IIA, IIB and IIX) are distinguished, in which specific isoforms of myosin heavy chains (MHC) are found (SCHIAFFINO and REGGIANI 1996, DEPREUX *et al.* 2002, EGGERT *et al.* 2002, CHANG *et al.* 2003, MELODY *et al.* 2004, DA COSTA and CHANG 2005, RYU and KIM 2005, CHOI *et al.* 2007, WOJTYSIK and MIGDAŁ 2007). Type I fibres are known as slow oxidative fibres, type IIB – as fast-twitch glycolytic fibres, while types IIA and IIX – as intermediate, oxidative glycolytic fibres. Electrophoretic separation of MHC isoforms in the analyzed porcine muscle (*m. longissimus thoracis et lumborum*) showed the presence of four types of MHC isoforms. At the same time, we need to stress here the unseparated bands of MHC isoforms types Ila and IIX (Figure). The above phenomenon was also observed in a study by MELODY *et al.* (2004).

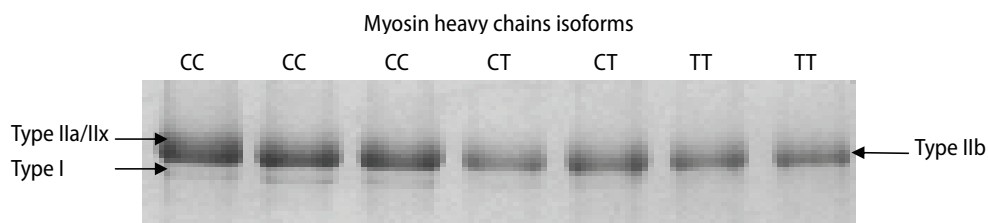


Figure
Electrophoretic separation of MHC isoforms in the *longissimus thoracis et lumborum* muscle obtained from pigs of different *RYR1* genotypes (8 % PAGE-SDS)

Elektrophoretische Trennung der MHC Isoformen im Schweinemuskel (*M. longissimus thoracis et lumborum*) mit verschiedenen *RYR1* Genotypen (8 % PAGE-SDS)

It results from earlier studies that individual types of fibres mature in a specific order (I ↔ IIA ↔ IIX ↔ IIB) (PETTE and STARON 1997) and later maturing fibers as well as fibres with a larger cross-section area are related, among things, with deteriorating meat quality (LEFAUCHEUR and GERRARD 2000). In this study a higher proportion of MHC isoforms type IIB was recorded in the group of pigs – *RYR1*^T carriers (TT and CT). At the same time meat sampled from that group of animals was characterized by inferior tenderness and water holding capacity in comparison to animals which did not carry the gene of susceptibility to stress. This probably resulted from an increased amount of fast

glycolytic fibres, as it was previously indicated also by other researchers (ESSÉN-GUSTAVSSON *et al.* 1992, DEPREUX *et al.* 2002, EGGERT *et al.* 2002, FIEDLER *et al.* 2001, WOJTYSIAK and MIGDAŁ 2007). At the same time they observed that the effect of fibre types on meat quality in pigs was more evident in the group of *RYR1*^T carriers than in the group of animals which did not carry that gene. Thus it became possible to use the composition of MHC isoforms in the selection of animals exhibiting susceptibility to stress (MARTENS 1998, DEPREUX *et al.* 2002, EGGERT *et al.* 2002, DA COSTA and CHANG 2005, LEFAUCHEUR 2006, CHOI *et al.* 2007).

Conducted studies showed a statistically significant ($P \leq 0.05$) effect of the *RYR1* gene on the proportion of isoforms of myosin heavy chains (MHC) type IIb (corresponding to fast glycolytic fibres) and type I (corresponding to slow oxidative fibres) in the muscle tissue of tested fatteners.

Carriers of the *RYR1*^T gene (TT and CT) were characterized by a significantly ($P \leq 0.05$) lower share of MHC isoforms type I and a higher proportion of MHC isoform type IIb in comparison to animals resistant to stress (CC).

Inferior tenderness (higher values of shear force in instrumental analysis and lower scores in sensory examination), as well as water holding capacity (bigger volume of centrifugal and thermal drip) of meat collected from pigs susceptible to stress (TT) at 144 h after slaughter could have been related, among other things, with increased contents of MHC isoforms type IIb.

The composition of MHC isoforms might be a useful indicator in breeding work in the selection of animals carrying the gene of susceptibility to stress.

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