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ANALYSIS OF THE CAUSES OF MEAT QUALITY VARIATIONS IN PIGS WITH IDENTICAL GENOTYPE

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The aim of the study was to evaluate causes of variations in the meat quality of pigs of the (Danish Landrace x Yorkshire) x Duroc [(LxY)xD] breed slaughtered at two different dates. Experiments were carried out on 36 pigs slaughtered in two groups of 18 animals each. The meat quality of the examined fatteners was evaluated on the basis of the analysis of the following parameters: pH value, electrical conductivity, changes of some selected proteins of washed myofibril fractions as well as its tenderness and water holding capacity. It was found that, even though the meat was characterised by normal quality, significant ($p \leq 0.05$) differences were observed in the examined parameters (pH measurements 48 and 144 h after slaughter, electrical conductivity 35 min and 3 h after slaughter, centrifugal and thermal drip, shear force and the share of titin degradation product T2 in the fraction of washed myofibrils, the share of MHC 2a isoform in the muscle tissue) between the compared groups of pigs. The observed variations in the muscle quality could have been caused by metabolic properties of muscle tissues as indicated by the proportion of the 2a myosin heavy chain (MHC) isoform as well as by different growth rates of experimental animals.

INTRODUCTION

Investigations which have been carried out so far concerning pork quality improvement focused mainly on the elimination from the animal breeding population of those individuals which were carriers of the stress susceptibility (RYR1) and acid meat (RN^-) genes. It was proved that the frequency of the occurrence of meat with quality defects was closely associated with genetic factors [Koćwin-Podsiadła, 1998a,b]. Other researchers [Karlsson *et al.*, 1999; Greaser *et al.*, 2001] reported a significant effect of muscle fibre types on meat quality, of which the fibre composition that could be helpful in determining relationships between muscle metabolic properties and the meat quality obtained from them deserve special attention [Chang *et al.*, 2003]. However, there are other elements influencing meat quality, including environmental factors associated with animal rearing conditions as well as their pre-slaughter handling [Łyczyński & Pospiech, 2003; Nienartowicz-Zdrojewska, 2004]. They can influence not only the rate of swine growth but, equally importantly, also the meat quality.

The aim of these studies was to assess causes of variations in the meat quality derived from pigs of the (Danish Landrace x Yorkshire) x Duroc [(LxY)xD] breed slaughtered at two different dates.

MATERIAL AND METHODS

The experimental material was the *longissimus thoracis* and *lumborum* muscles collected from 36 pigs of the (L x Y) x D breed at two different (not very much apart) dates of slaughter of two groups of 18 animals each. All the experimental animals were free from the stress susceptibility gene RYR1.

Meat quality evaluation of the experimental fatteners was carried out employing the following methods: pH value measurement (45 min, 24, 48 and 144 h after slaughter using the Handylab 2 type pH-meter equipped in a combine electrode, of the Schott L68880 type) according to Polish Norm [PN-ISO 2917 2001]; measurement of the electrical conductivity value (35 min, 3 and 24 h after slaughter using the LF STAR Company equipment) [Strzelecki *et al.*, 1995]; electrophoretic analysis (in 8% PAGE-SDS with urea according to Pospiech *et al.* [2000a]) of selected proteins obtained from washed myofibrils 45 min, 24, 48 and 144 h after slaughter; composition evaluation of the myosin heavy chain (MHC) isoforms (in 8% PAGE-SDS according to Mozdziak *et al.* [1998]) obtained from the fraction of washed myofibrils 45 min after slaughter; instrumental measurement of meat tenderness (using the Instron 1140 type apparatus with the Warner-Bratzler device – according to Dobrzycki [1990])

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TABLE 1. Effect of selected parameters on meat quality of pigs of the (LxY)xD breed slaughtered at different dates.

Evaluated parameters	Term of slaughter	
	first	second
pH value		
45 min	6.6^{a} ±0.19	6.6^{a}±0.17
24 h	5.76^{b}±0.08	5.66^{a}±0.14
48 h	5.53^{a}±0.10	5.74^{b}±0.14
144 h	5.61^{a}±0.11	5.76^{b}±0.23
Electrical conductivity (mS/cm)		
35 min	3.54^{a}±0.34	4.42^{b}±1.13
3 h	3.85^{b}±0.62	2.81^{a}±0.68
24 h	4.12^{a}±0.78	4.69^{a}±1.19
Centrifugal drip (%)		
48 h	21.69^{b}±2.53	18.75^{a}±3.09
144 h	15.54^{b}4.17	11.79^{a}±4.38
Thermal drip (%)		
48 h	31.61^{b}±2.51	17.88^{a}±2.41
144 h	32.47^{b}±2.32	15.50^{a}±2.30
Shear force (N/cm^2)		
48 h	78.46^{b}±13.47	43.96^{a}±5.44
144 h	31.16^{a}±5.77	36.43^{b}±3.70
Content of titin degradation product T2 in the fraction of washed myofibrils (%)		
45 min	2.83^{b}±1.02	2.02^{a}±0.87
24 h	2.48^{a}±0.65	2.79^{a}±0.84
48 h	4.16^{b}±0.62	3.38^{a}±0.96
144 h	4.75^{b}±0.86	4.00^{a}±1.10
Content of MHC 2a isoform in the muscle tissue (%)	21.21^{a}±13.13	34.30^{b}±15.54

$^{a.\ b...}$ mean values from the same row having various letters differ statistically significantly at $p \leq 0.05$

and measurement of meat water holding capacity (on the basis of thermal losses and the size of the centrifugal drip) 48 and 144 h after slaughter.

The measurements of the pH value and electrical conductivity in the examined muscles were taken at the height of the last thoracic vertebra of the left half carcasses.

The fraction of washed myofibrils was obtained after a double extraction of the muscle tissue using the rigor buffer (75 mmol/L KCl, 10 mmol/L KH_2PO_4, 2 mmol/L $MgCl_2$, 2 mmol/L EGTA of pH 7.0, and 0.1 mol/L PMSF) [Fritz *et al.*, 1989]. The analytical sample of 4 mg of washed myofibrils was suspended in 100 µL of buffer A (8 mol/L urea, 2 mol/L tiourea, 0.05 mol/L Tris o pH 6.8, 75 mmol/L DTT, 3% SDS, 0.05% bromophenol blue) to allow solubilization. Next, samples were heated at a temperature of 100°C for three minutes. The size of the analytical sample to be transferred onto the gel ranged from 4 to 8 µL. Protein separation of the washed myofibril fraction was performed vertically using the Hoefer Scientific Instruments apparatus of SE 250 (for the analysis of protein in 8% PAGE-SDS with urea) and SE 260 (for the analysis of the MHC isoforms in 8% PAGE-SDS) types. Both quantitative and qualitative assessment of the separated proteins of the washed myofibril fraction was carried out using a scanning densitometer, type Image Master® VDS. The percentage of individual protein bands was estimated in relation to all bands occurring in a given sample.

The instrumental assessment of meat tenderness consisted in the determination of the maximal force necessary to shear meat samples measuring 10 x 10 x 40 mm, with fibres running perpendicular to the cutting plane. This parameter was evaluated following the thermal treatment of meat slices 25 to 300 mm thick in a convection furnace type Rational Combi in hot air of 160°C for about 15 min until the temperature in the sample centre reached 72°C.

Thermal losses were determined from weight differences before and after the heating of meat slices weighing 120 to 160 g.

The centrifugal drip was obtained by spinning 6 g of the muscle tissue (for 20 min at 25480xg) in the SIGMA Company centrifuge type 3K30.

All the examined parameters of meat quality evaluation were subjected to the analysis of variance (ANOVA) using the STATISTICA 6.0 software package. The significance of differences between groups of mean values ($p \leq 0.05$) was determined using the Fischer test [Stanisz, 1998].

RESULTS AND DISCUSSION

The analysis of classical quality indices (pH and electrical conductivity values) revealed that the examined meat, irrespective of the date of slaughter, showed normal quality properties (Table 1). Differences in the pH value of the meat from the two dates of slaughter amounted to 0.1–0.2 pH unit and were statistically significantly ($p \leq 0.05$) different from the 24-h of cold storage. The meat raw material derived from the second date of slaughter was characterized by higher pH values (Table 1).

Statistically significant ($p \leq 0.05$) differences in the values of electrical conductivity (EC) were observed 35 min (3.54 mS/cm in the first and 4.42 mS/cm in the second slaughter date) and 3 h (3.85 mS/cm in the first and 2.81 mS/cm in the second slaughter date) after slaughter (Table 1).

The analysis of the water holding capacity showed statistically significant ($p \leq 0.05$) differences in the size of thermal losses and the centrifugal drip of the obtained pork meat at two different dates of slaughter (Table 1). Significantly lower thermal losses (17.88% - 48 h and 15.50% - 144 h after slaughter) as well as smaller drips (18.75% - 48 h and 11.79% - 144 h after slaughter) were recorded for the meat of pigs slaughtered later (Table 1).

A significantly lower ($p \leq 0.05$) value of the shear force, *i.e.* better tenderness, 48 h after slaughter was found in the meat of pigs slaughtered at the second date (43.96 N/cm^2), whereas 144 h after slaughter – in the meat from pigs slaughtered earlier (31.16 N/cm^2) (Table 1). It should be mentioned here that 5 days after cold storage, the values of shear forces corresponded to those of tender meat irrespective of the observed differences. The comparison of the results of meat tenderness assessment with the titin T2 changes (one of the largest products of the native titin degradation) showed that a significantly ($p \leq 0.05$) higher proportion of this protein was observed in the fraction of washed myofibrils derived from the meat of pigs slaughtered earlier (4.16% - 48 h and 4.75% - 144 h) than in the meat of pigs slaughtered later (3.38% - 48 h and 4.00% - 144 h) (Table 1). The above-mentioned observations may indicate that the protein degradation processes responsible for tissue tenderness and water holding capacity were more advanced in the muscles of pigs slaughtered later. In analysing variations in the meat tenderness and water holding capacity, it is worth emphasizing that, apart from the tissue proteolysis which could have affected these properties, another important factor influencing them could have been glycolytic changes and their rate. The higher pH value recorded at the later dates of analyses of this meat could have contributed to its higher water holding capacity (lower centrifugal and thermal losses) and tenderness (smaller shear force) (Table 1).

The employed electrophoresis (in 8% PAGE-SDS) identified 4 types of isoforms of myosin heavy chains (MHC). Three of them corresponded to the fast- (MHC-2a, 2x and 2b) and one (MHC I) - to the slow-contracting (oxidative) fibres. In this case, statistically significant ($p \leq 0.05$) differences were found only in the percentage of the MHC 2a isoform which corresponds to the fast contracting, oxidative-glycolytic fibres. The significantly ($p \leq 0.05$) higher proportion of this isoform was recorded in the muscle tissue derived from pigs from the second slaughter date (34.30%) when compared with the first one (21.21%) (Table 1). A characteristic property of these fibres is that they enter the contraction reaction faster than fibres of the I type, slow-contracting ones. The observed higher water holding capacity of the meat from the second slaughter date could, therefore, have been associated with the increased proportion of the above-mentioned isoform (Table 1).

There are several factors which can modify the rate of *post mortem* changes in the muscle tissue [Bee *et al.*, 2004; Rosenvold & Andersen, 2003; Hamilton *et al.,* 2003; Moeller *et al.*, 2003; Pösö & Puolanne, 2005]. The genetic factors and those associated with morphology of muscle fibers belong to the most important ones [Plastow *et al.,* 2005]. They determine the direction and the size of the glycolytic and the proteolytic changes in meat. In opinion of several authors [Kaufman, 1996; Plastow *et al.*, 2005], the environmental factors become dominant when the mutations causing the appearance of meat defects are eliminated. They are associated with animal feeding, its size and type, and/or with animal rearing conditions. Mozdziak *et al*. [1998] observed the changes in the ratio of various myosin heavy chains by the restriction of animal moving, which indicate the changes in muscle metabolism. The number of glycolytic forms of MHC increased. The enforcement of pigs to moving during their feeding caused reversed reaction [Jørgensen & Hyldgaard-Jensen, 1975].

Results obtained in this study indicate that the observed variations in the meat quality of pigs derived from the same breeding group can be attributed to different metabolic properties of their muscle fibers. The interval of two weeks in the term of their slaughter, despite the fact, that they were slaughtered at the same body weight, arose from the different rate of their growth, which could be related with the change in the metabolism of muscles. This could have resulted in different meatiness of pigs [Pospiech *et al.,* 2000b] or as in this case the change in meat quality. Similar relationship was observed by the change of the slaughter weight of pigs [Pospiech *et al.,*1983].

CONCLUSIONS

1. Meat obtained from pigs of the (Danish Landrace x Yorkshire) x Duroc [(LxY)xD] breed which were slaughtered at different dates differed significantly with regard to the pH value (24, 48 and 144 h after slaughter) and electrical conductivity (35 min and 24 h after slaughter) as well as tenderness and water holding capacity evaluated on the basis of the centrifugal drip and thermal losses.
2. Meat derived from pigs slaughtered at the later date was characterised by a markedly better tenderness 48 h after slaughter, whereas the meat from the first slaughter date exhibited significantly lower values of the shear force 144 h after slaughter.
3. The improved meat tenderness after 144 h cold storage was accompanied by an increased proportion of titin Tl degradation products obtained from the fraction of washed myofibrils.
4. The levels of thermal losses as well as of the drip obtained from the meat of the experimental animals in the case of the second slaughter date were significantly lower in comparison with the first date.
5. The significantly higher water holding capacity of the meat from pigs slaughtered at the second date could have been connected with the increased proportion of the 2a MHC isoform corresponding to the fast-contracting, oxidative-glycolytic fibres.
6. The observed variations in the muscle tissue quality could have resulted from different rates of growth of the experimental animals.

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ANALIZA PRZYCZYN ZRÓŻNICOWANEJ JAKOŚCI MIĘSA POZYSKANEGO OD ŚWIŃ O TYM SAMYM GENOTYPIE

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Celem pracy była ocena przyczyn zróżnicowanej jakości mięsa pozyskanego świń od rasy (Landrace duńska x Yorkshire) x Duroc [(LxY)xD] poddanych ubojowi w dwóch odrębnych terminach uboju. Badania przeprowadzono na 36 świniach poddanych ubojowi w dwóch grupach. w każdej po 18 sztuk. Jakość mięsa badanych tuczników oceniono na podstawie analizy wartości pH, przewodności elektrycznej, przemian wybranych białek frakcji przemytych miofibryli oraz jego kruchości i wodochłonności. Stwierdzono, że jakkolwiek mięso wykazywało cechy normalnej jakości to jednak wystąpiły istotne ($p \leq 0.05$) różnice w wartości pH mierzonej po 48 i 144 godz. od momentu uboju, przewodności elektrycznej po 35 min i 3 godz. po uboju, wielkości wycieku wirówkowego i termicznego oraz siły cięcia a także udziału produktu degradacji titiny T2 we frakcji przemytych miofibryli i izoformy MHC 2a w tkance mięśniowej pomiędzy porównywanymi grupami świń. Zróżnicowanie w ich jakości mogło wynikać z różnych właściwości metabolicznych włókien mięśniowych, co stwierdzono na przykładzie udziału izoformy MHC 2a, a także z różnej szybkości wzrostu świń.