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COMPARISON OF POLYMORPHIC MYOSIN FORMS WITH SELECTED MEAT QUALITY ATTRIBUTES OF BULLS OF VARIOUS BREEDS AND AGE

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The aim of the study was to compare the shares of MHC isoforms in the muscle tissue of bulls differing in terms of breed and age using SDS-PAGE. The share was analysed as a factor which could have modified tenderness and water holding capacity of meat. Investigations were conducted on 73 young bulls of the Black-and-White, Polish Red, Hereford and Limousine breeds, slaughtered at the age of 6, 9 and 12 months, generally with 5 animals in each age group. The composition of MHC isoforms (type 2a, 2x and I) in the muscle tissue of the analysed cattle was related to the instrumental tenderness evaluation, as well as water holding capacity of meat on the basis of drip loss at 48, 96 and 240 h after slaughter. It was shown that better tenderness and water holding capacity of meat from Hereford bulls aged 12 months were accompanied by an increased share of MHC isoform type 2a, corresponding to fast-twitch, intermediate, oxygen glycolytic fibers.

INTRODUCTION

Production of culinary beef in countries of Western Europe is based on cattle of beef breeds. At the same time in Poland meat from native cattle breeds predominates, especially dairy or dairy-beef Black-and-White cattle, usually of low quality Jasiorowski, 2000; Grześkowiak *et al.,* 2006]. Commercial crossing of cows of dairy breeds with bulls of beef breeds, such as *e.g.* Hereford or Limousine, is promoted in order to improve eating quality of Polish beef [Kołczak, 2000; Litwińczuk, 2004; Wajda *et al.,* 2004; Grodzki, 2005]. These breeds have a considerable genetic potential and their meat, as it has already been shown in many studies, exhibits good quality in spite of low fatness [Litwińczuk *et al.,* 2001; Daszkiewicz & Wajda, 2002; Wajda *et al.,* 2004]. Apart from genotype, the age of animals has a significant effect on beef quality [Wulf *et al.,* 1996; Kołczak *et al.,* 2003; Grodzki, 2005; Litwińczuk & Szulc, 2005]. At present meat from young cattle slaughtered at the age of 8-12 months became the focus of interest of breeders, since potentially it is the source of raw material with best cooking value [Pisula *et al.,* 2007]. One of the most crucial quality attributes of beef in consumer perception is tenderness. In recent years a significant relationship of metabolic properties of skeletal muscle fibers, assessed on the basis of myosin heavy chain (MHC) polymorphic forms, with the tenderisation process is emphasized with increasing frequency [Karlsson *et al.,* 1999; Piccard *et al.,* 1999; Eggert *et al.,* 2002; Greaser *et al.,* 2001; Chang *et al.,* 2003; Chikuni *et al.,* 2004].

The aim of the study was to compare, using SDS-PAGE, the share of MHC isoforms in the muscle tissue of bulls differing in terms of their breed and age. The share of these isoforms was analysed as a factor which might modify tenderness and water holding capacity of meat.

MATERIAL AND METHODS

The experimental material was the chest and lumbar part of the longest muscle (*m. longissimus thoracis et lumborum*) collected from a total of 73 half-carcasses of slaughtered young bulls. The analysis was conducted on bulls of four breeds: Black-and-White (n=22), Polish Red (n=19), Hereford (n=15) and Limousine (n=17), aged 6, 9 and 12 months, as a rule with 5 animals in each age group. Animals were raised at the experimental station of the Institute of Genetic and Animal Breeding of Polish Academy of Science in Jastrzębiec. Their slaughter was conducted at the slaughter house of this Institute. Next day, after 24 h carcasses' chilling, the longest muscle was divided on three the same parts for investigation at 48, 96 and 240 h. After that they were transported in separated plastic bags at cooled conditions to the laboratory, where they were vacuum packed and kept at 2–4°C. Samples assigned for the quality evaluation and electrophoretic study were taken from the same part of the muscle in fixed term of investigations. Electrophoretic samples were frozen in liquid nitrogen and stored until investigation at -81°C. Meat from the analysed animals exhibited attributes of normal quality.

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The share of myosin heavy chain (MHC) isoforms in the fraction of washed myofibrils from the muscle tissue 45 min after slaughter was analysed using electrophoresis in 8% PAGE-SDS [Mozdziak *et al.,* 1998]. The fraction of washed myofibrils was obtained as a result of double extraction of the muscle tissue with a phosphate buffer solution, the so-called rigor buffer (75 mmol KCL, 10 mmol KH_2PO_4, 2 mmol $MgCl_2$, 2 mmol EGTA with pH 7.0, 0.1 mol PMSF) [Fritz *et al.,* 1989]. Proteins of this fraction were separated in the vertical system on double-layer polyacrylamide gel with SDS in an SE 260 apparatus by Hoefer Scientific Instruments. The upper layer consisted of stacking gel containing 2.56% acrylamide, 0.45% N,N'-diallytartardiamide (DATD), 10% glycerol, 0.1 mol Tris-HCl (pH 6.8), 0.1% SDS, 0.05% ammonia persulfate and 0.5% N,N,N',N'–tetramethylethylenediamine (TEMED). The bottom layer consisted of separating gel containing 29.4% acrylamide, 0.6% N,N'--methylenebisacrylamide, 30% glycerol, 0.2 mol Tris (pH 8.8), 0.1 mol glycine, 0.4% SDS, 0.03% ammonium persulfate and 0.1% TEMED. The final concentration of acrylamide in the separating gel was 8%. Each sample transferred onto gel contained 0.5 μg of protein. Electrophoretic separations were run for 24 h at a constant voltage of 70 V at 4°C. After the completion of separations gels were stained in a solution containing 0.05% Coomassie Blue R-250, 45% methanol and 9.2% acetic acid and next destained in a mixture of 10% methanol and 7.5% acetic acid. Quantitative analysis of separated bands of MHC isoforms was performed in an Image Master® VDS scanning densitometer of Pharmacia using the Image Master™ 1D Elite version 4.00 software. Calculations were based on the assumption that the area of a single protein band constitutes its percentage in relation to the areas of all separated proteins in a given sample on gel, which amount to 100%.

Meat tenderness evaluated instrumentally and expressed as the maximum shear force was measured using a 1140 Instron testing machine with a Warner-Bratzler shear attachment, after previous thermal treatment of the sample. Slices with the thickness of 25-30 mm were heated in a water bath at 80-81°C. After reaching the temperature of 72°C inside slices, samples were kept in a water bath of the same temperature for the next 90 min. After cooling cuboids with the cross section of 10 mm x 10 mm and length of approx. 40 mm were cut from slices taking into consideration the parallel arrangement of muscle fibers. Samples were subjected to the action of shear force so that the arrangement of fibers was perpendicular to the shear plane.

Water holding capacity of meat was assessed on the basis of the volume of drip loss, established from the difference of weight of meat pieces (with mean weight of 700-900 g) before and after storage at 4°C.

Instrumental evaluation of tenderness and measurements of water holding capacity of meat were conducted 48, 96 and 240 h after slaughter.

Results of studies were subjected to the two-way analysis of variance (ANOVA) using Statistica 6.0 PL software. The significance of differences between groups of means ($p \leq 0.05$) was determined using the Fischer test [Stanisz *et al.,* 1998].

RESULTS AND DISCUSSION

In skeletal muscles of adult mammals four types of fiber may be distinguished: 2a, 2x, 2b and I, in which respective myosin heavy chain (MHC) isoforms are expressed [Bottinelli, 2001]. The first three types of fiber belong to the class of fast twitch fibers, either oxygen glycolytic (types 2a and 2x) or glycolytic (type 2b), while the last type, *i.e.* I, corresponds to slow twitch oxygen fibers. In the analysed beef the presence of only three types of MHC isoforms: 2a, 2x and I, was detected by electrophoresis in 8% PAGE-SDS (Figures 1 and 2). Isoform type 2b was not observed. The absence of this isoform in bovine muscles was also reported by other researchers [Chikuni *et al.,* 2004], who suggested that it may prove useful in the explanation of quality differences between beef and pork.

Statistical analysis of the share of MHC isoforms in the muscle tissue of the analysed bulls showed the highest number of significant ($p \leq 0.05$) differences in the case of MHC isoform type 2a (Table 1). In terms of the metabolic type it is closer to fibers type I of oxygen character [Chang *et al.,* 2003]. The highest percentages of this isoform were recorded in meat of Limousine bulls (41.26-53.94%), while the lowest in meat of Black-and-White (20.00-33.06%). The muscle tissue from Hereford and Limousine, irrespective of age, exhibited a higher content of MHC isoform type 2a in comparison to Black-and-White and Polish Red bulls. An analysis of the share of type 2x MHC isoform, closer to the anaerobic fibers type 2b, showed a significant ($p \leq 0.05$) differentiation between cattle of the Black-and-White and Polish Red slaughtered at the age of 6 months (21.59% and 38.44%, respectively) and also between cattle of the Black-and-White 6 months old and Limousine 12 months old (21.59% and 34.52% respectively). In turn, the share of MHC isoform type I was significantly ($p \leq 0.05$) higher in the muscle tissue collected from Black-and-White (41.51-58.42%) in comparison to the Limousine bulls (19.04-24.03%) (Table 1).

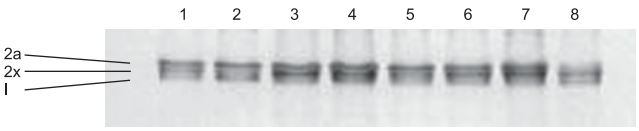

FIGURE 1. The separation of MHC isoforms from beef of Black-and-White (BW) and Polish Red (PR) bulls in 8% PAGE-SDS (band 1 – sample collected from BW bulls slaughtered at the age of 6 months; band 2 – sample from BW 9 months; bands 3 and 4 – samples from BW 12 months; band 5 – sample from PR 6 months; band 6 – sample from PR 9 months; bands 7 and 8 samples from PR 12 months).

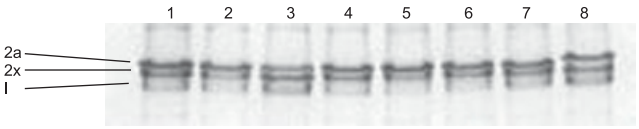

FIGURE 2. The separation of MHC isoforms from beef of Hereford and Limousine bulls in 8% PAGE-SDS (band 1 – sample collected from Hereford bulls slaughtered at the age of 6 months; band 2 – sample from Hereford 9 months; bands 3 and 4 – samples from Hereford 12 months; band 5 – sample from Limousine 6 months; band 6 – sample from Limousine 9 months; bands 7 and 8 samples from Limousine 12 months).

TABLE 1. Effect of breed and age of bulls on the share of MHC isoforms in muscle tissue.

Breed	Age (months)	N	Share of MHC isoforms (%)		
			Type 2a	Type 2x	Type I
Black-and-White	6	4	$20.00^{a}\pm1.34$*	$21.59^{a}\pm7.35$	$58.42^{f}\pm8.35$
	9	6	$22.96^{ab}\pm3.77$	$25.82^{abc}\pm7.43$	$51.22^{ef}\pm8.95$
	12	12	$33.06^{d}\pm5.64$	$25.43^{ab}\pm6.65$	$41.51^{d}\pm9.61$
Polish Red	6	5	$32.41^{cd}\pm3.76$	$38.44^{e}\pm5.48$	$29.15^{abc}\pm3.87$
	9	5	$27.87^{bc}\pm3.89$	$33.16^{cde}\pm2.73$	$38.97^{cd}\pm5.55$
	12	9	$25.31^{ab}\pm5.31$	$32.11^{cde}\pm8.67$	$42.58^{de}\pm8.54$
Hereford	6	5	$47.02^{g}\pm2.11$	$29.29^{abcd}\pm2.31$	$23.69^{ab}\pm3.68$
	9	5	$35.28^{de}\pm3.23$	$31.38^{bcde}\pm6.94$	$33.35^{bcd}\pm9.91$
	12	5	$39.42^{ef}\pm6.17$	$28.21^{abcd}\pm9.32$	$32.37^{bc}\pm11.91$
Limousine	6	5	$53.94^{h}\pm6.32$	$27.02^{abcd}\pm4.82$	$19.04^{a}\pm6.22$
	9	5	$46.26^{g}\pm5.84$	$29.71^{abcd}\pm4.30$	$24.03^{ab}\pm5.80$
	12	7	$41.56^{fg}\pm5.17$	$34.52^{de}\pm7.95$	$23.92^{ab}\pm10.06$

$^{a.b}$ – means in the same column followed by different letters are significantly different ($p\leq0.05$); * – standard deviation

TABLE 2. Effect of breed and age of cattle on the WBSF values (N/cm^2) from instrumental evaluation of tenderness.

Breed	Age (months)	N	Time of *post mortem* meat aging (h)		
			48	96	240
Black-and-White	6	4	$140.85^{abc}\pm5.18$*	$124.75^{ab}\pm14.81$	$124.33^{b}\pm18.28$
	9	6	$144.43^{abc}\pm15.03$	$138.16^{ab}\pm16.74$	$103.12^{ab}\pm35.27$
	12	12	$149.58^{abc}\pm26.78$	$122.19^{ab}\pm42.88$	$103.43^{ab}\pm27.93$
Polish Red	6	5	$164.62^{bc}\pm6.72$	$147.34^{b}\pm5.14$	$111.11^{ab}\pm21.68$
	9	5	$170.29^{c}\pm10.33$	$145.15^{b}\pm7.22$	$116.27^{ab}\pm10.08$
	12	9	$141.24^{ab}\pm26.20$	$137.25^{b}\pm15.60$	$112.81^{ab}\pm23.78$
Hereford	6	5	$159.21^{bc}\pm29.29$	$136.15^{ab}\pm24.23$	$110.55^{ab}\pm46.27$
	9	5	$143.92^{abc}\pm18.09$	$129.16^{ab}\pm22.55$	$106.73^{ab}\pm20.96$
	12	5	$145.77^{abc}\pm24.68$	$138.23^{ab}\pm31.38$	$95.85^{ab}\pm14.97$
Limousine	6	5	$130.67^{a}\pm34.83$	$109.41^{a}\pm21.39$	$84.56^{a}\pm24.83$
	9	5	$151.95^{abc}\pm19.96$	$143.93^{b}\pm5.12$	$99.27^{ab}\pm21.02$
	12	7	$152.77^{abc}\pm16.84$	$141.67^{b}\pm16.00$	$120.28^{b}\pm17.57$

$^{a.b}$ – means in the same column followed by different letters are significantly different ($p\leq0.05$); * – standard deviation

TABLE 3. Effect of breed and age of cattle on value of drip loss (%) from muscle tissue.

Breed	Age (months)	N	Time of *post mortem* meat aging (h)		
			48	96	240
Black-and-White	6	4	$1.24^{bc}\pm0.22$*	$1.58^{abcd}\pm0.52$	$2.09^{ab}\pm1.12$
	9	6	$1.24^{bc}\pm0.97$	$1.65^{cd}\pm0.94$	$2.28^{ab}\pm0.76$
	12	12	$0.64^{a}\pm0.48$	$1.00^{ab}\pm0.38$	$1.92^{a}\pm0.91$
Polish Red	6	5	$1.26^{bc}\pm0.14$	$1.62^{abcd}\pm0.41$	$1.89^{ab}\pm0.43$
	9	5	$1.59^{c}\pm0.35$	$1.46^{abcd}\pm0.42$	$2.09^{ab}\pm0.62$
	12	9	$0.53^{a}\pm0.36$	$0.95^{a}\pm0.37$	$1.92^{a}\pm1.08$
Hereford	6	5	$0.96^{abc}\pm0.43$	$1.37^{abcd}\pm0.65$	$2.08^{ab}\pm0.66$
	9	5	$1.21^{bc}\pm0.61$	$1.54^{abcd}\pm0.51$	$1.54^{a}\pm0.59$
	12	5	$0.91^{ab}\pm0.36$	$1.23^{ac}\pm0.46$	$1.54^{a}\pm0.63$
Limousine	6	5	$0.99^{abc}\pm0.74$	$2.10^{d}\pm1.05$	$3.67^{c}\pm1.09$
	9	5	$0.99^{abc}\pm0.58$	$1.37^{abcd}\pm0.75$	$2.88^{bc}\pm0.72$
	12	7	$1.02^{abc}\pm0.45$	$1.62^{cd}\pm0.58$	$2.25^{ab}\pm0.61$

$^{a.b}$ – means in the same column followed by different letters are significantly different ($p\leq0.05$); * – standard deviation

Analysis of tenderness on the basis of WBSF measurement at 48 h after slaughter showed statistically significant ($p\leq0.05$) differences in values of shear force between meat of Limousine (130.67 N/cm^2 for bulls in age of 6 months) and Hereford (159.21 N/cm^2 for bulls of the same age) and Polish Red (164.62 N/cm^2 for bulls of the same age and 170.29 N/cm^2 for bulls 3 months older). Meat obtained from bulls of the Limousine breed in age of 6 months exhibited also the lowest values of shear force, *i.e.* the best tenderness at the 96 h (109.41 N/cm^2) and 240 h (84.56 N/cm^2) after slaughter (Table 2). However, in cattle of this breed meat tenderness deteriorated with age. Among the analyzed breeds of cattle slaughtered in age of 12 months the best tenderness in instrumental evaluation was recorded for meat from Hereford bulls (95.85 N/cm^2). In contrast, the worst tenderness was observed in meat from Limousine breed (120.28 N/cm^2) (Table 2).

Analysis of water holding capacity at 48 h after slaughter showed significantly ($p \leq 0.05$) lower drip losses in meat of Black-and-White and Polish Red bulls aged 12 months (0.64% and 0.53%, respectively) in comparison to those aged 6 months (1.24% and 1.26%) and 9 months (1.24% and 1.59%, respectively) (Table 3). At 96 h after slaughter the volume of drip losses was significantly ($p \leq 0.05$) different between Polish Red bulls aged 12 months (0.95%) and Limousine bulls aged 6 (2.10%) and 12 months (1.62%), as well as Limousine bulls aged 6 and 12 months and Black-and-White bulls aged 12 months (1.00%). In contrast, at 240 h after slaughter statistically significant ($p \leq 0.05$) differences in the volume of drip losses were observed between Hereford bulls aged 9 and 12 months (1.54%) and Limousine bulls aged 6 (3.67%) and 9 months (2.88%), (Table 3). In this case the lowest drip losses, *i.e.* the best water holding capacity, were found for meat produced by Hereford bulls aged both 9 and 12 months.

Results of the study showed the best tenderness, as well as water holding capacity of meat at 240 h after slaughter in the case of Hereford bulls aged 12 months (Tables 2 and 3). The main advantage of this small British beef breed of meat purpose is its ability to adapt to difficult harsh climatic conditions and extensive feeding [Litwińczuk & Szulc, 2005]. Much worse results of eating and technological value assessment were recorded for meat produced by other breeds of beef cattle, *i.e.* Limousine. Popularity and high opinion on this large French breed result from the excellent value of their carcasses and meat [Litwińczuk & Szulc, 2005]. In a study by Dikeman *et al.* [2005] it was pointed out that meat of the Limousine breed reaching a higher body weight exhibits lower marbling and thus *e.g.* inferior tenderness in comparison to the Hereford breed. Generally it is believed that beef cattle breeds are characterized by better meat quality than dairy cattle [Wajda *et al.,* 2004]. Identical results were observed in this study. At the same time it was found that the best quality attributes of meat produced by Hereford bulls slaughtered in age of 12 months were accompanied by increased contents of MHC isoform type 2a, corresponding to fast twitch, intermediate, oxygen glycolytic fibers. Increased amount of these fibers in the muscle tissue may determine high quality of meat by improving its tenderness and water holding capacity.

CONCLUSIONS

1. The muscle tissue obtained from the Hereford and Limousine bulls was characterized by a significantly ($p \leq 0.05$) higher content of MHC isoform type 2a in comparison to the Black-and-White and Polish Red bulls.

2. The best tenderness, assessed by the measurements of maximum shear force, at 240 h after slaughter was recorded for meat of Limousine bulls slaughtered in age of 6 months and Hereford bulls of 12 months old.

3. The best water holding capacity, assessed on the basis of drip loss at 240 h after slaughter, was found for meat of Hereford bulls slaughtered in age of 9 and 12 months.

4. Better tenderness and water holding capacity of meat from Hereford bull calves aged 12 months were accompanied by an increased share of MHC isoform type 2a, corresponding to fast twitch, intermediate, oxygen glycolytic fibers.

5. Increased amount oh MHC type 2a isoform in the muscle tissue may effect better quality of meat by improving its tenderness and water holding capacity.

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PORÓWNANIE FORM POLIMORFICZNYCH MIOZYNY Z WYBRANYMI WSKAŹNIKAMI JAKOŚCI MIĘSA BYCZKÓW ZRÓŻNICOWANYCH POD WZGLĘDEM RASY I WIEKU

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Celem pracy było porównanie udziału izoform MHC w tkance mięśniowej byczków zróżnicowanych pod względem rasy i wieku za pomocą techniki SDS-PAGE. Ich udział analizowano jako czynnik, który mógł oddziaływać na kształtowanie kruchości i wodochłonności mięsa. Badania przeprowadzono na 73 sztukach młodego bydła rasy czarno-białej, polskiej czerwonej, Hereford i Limousine poddanych ubojowi w wieku 6, 9 i 12 miesięcy, z reguły po 5 sztuk z każdej grupy wiekowej. Skład izoform MHC (typu 2a, -2x i -I) w tkance mięśniowej badanego bydła odniesiono do instrumentalnej oceny kruchości, a także wodochłonności mięsa przeprowadzonej na podstawie wielkości wycieku naturalnego po 48, 96 i 240 h od momentu uboju. Wykazano, że lepszej kruchości i wodochłonności mięsa pozyskanego od buhajków rasy Hereford w wieku 12 miesięcy towarzyszył zwiększony udział izoformy MHC typu 2a, odpowiadającej włóknom szybko kurczliwym, pośrednim, oksydatywno-glikolitycznym.