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Changes in proteins and tenderness of meat from young bulls of four breeds at three ages over 10 days of cold storage*

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The study aimed at analysing the changes taking place in the muscle tissue of cattle during meat cold storage in relation to animal genotype (breed) and age. Investigations were conducted on the thoracic and lumbar part of the *longissimus dorsi* (LD) muscle of Polish Holstein-Friesian (PHF) Black-and-White variety, Polish Red (PR), Hereford (H) and Limousine (L) bulls slaughtered at the age of 6, 9 and 12 months. Muscle analyses were carried out 45 min and 48, 96 and 240 h post-slaughter, separating proteins with the assistance of SDS-PAGE (electrophoresis in polyacrylamide gel using SDS). To identify titin, desmin and troponin T (Tn-T), their antibodies and western blotting were employed. The breed occurred to be the key factor affecting proteins' changes in the muscle tissue. The process of protein degradation in PHF was similar to that found in H while in PR – to that occurring in L bulls, despite the genotype differences between them. The greatest differences

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in protein changes were found between the meat obtained from bulls at the age of 6 *vs*. 12 months. During meat cold storage, day 10 turned out to be critical with regard to the degradation of almost all proteins. The drop of the content of titin, desmin and Tn-T was observed, simultaneously with the increase in their degradation products. The highest myofibrillar protein degradation observed on day 10 of cold storage proves these changes.

KEY WORDS: beef / tenderness / myofibrillar proteins / proteolysis / SDS-PAGE / immunoblotting

Tenderness belongs to the most important meat quality traits [Ouali 1990, Koohmaraie 1992, 1996, Miller *et al.* 1995, 2001, Huffman *et al.* 1996, Koohmaraie and Geesink 2006] and particularly of culinary beef [Grunert 1997, Houbak *et al.* 2008].

The rate of meat proteolysis depends especially on animal age and genotype, meat content of carcass and meat chemical composition. The course of proteolysis process that promotes the development of ageing of meat depends on the structure of two main meat components, *i.e.* myofibrillar and intramuscular connective tissue proteins [Nishimura *et al*. 1999, Sawdy *et al.* 2004, Koohmaraie and Geesink 2006] as well as on muscle metabolism and meat treatment before consumption. Ageing changes taking place in the myofibrillar structure include destabilization of the sarcomere Z disc and degradation of cytoskeletal and some myofibrillar regulatory proteins [Huff-Lonergan *et al.* 1995, Taylor *et al.* 1995, Boyer-Berri and Greaser 1998, Schmidt 1999, Kristensen and Purslow 2001, Kołczak *et al.* 2003ab]. Tenderness is usually associated with cytoskeletal degradation processes of titin, nebulin and desmin [Huff-Lonergan *et al*. 1995, Steen *et al*. 1997, Koohmaraie and Geesink 2006]. Many authors [Penny and Dransfield 1979, Ho *et al.* 1994, Koohmaraie 1994, Negishi *et al*. 1996, O'Halloran *et al.* 1997] relate the process of meat ageing to proteolysis of Tn-T and the appearance of fragments of molecular weight of 30 kDa. This protein is often accepted as a good indicator of meat tenderization process.

The aim of this study was to assess the changes occurring in myofibrillar proteins content of the muscle tissue of young bulls during a 10-day meat ageing process in a cold store. In addition, the relationship between the meat proteolytic changes and tenderness was determined as expressed by the shear force.

Material and methods

Material

Observations were carried out on the thoracic and lumbar part of the *longissimus dorsi* (LD) muscle of young bulls of the following four breeds: Polish Holstein-Friesian (PHF) Black-and-White variety, Polish Red (PR), Hereford (H) and Limousine (L) slaughtered at the age of 6, 9 and 12 months. Animals originated from the farm of the Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzębiec. Housing and feeding (individual) were the same for all bulls. Muscle samples for

analyses were collected from a total of 59 animals. The number of bulls in individual groups taking into account the breed and age at slaughter ranged from 5 to 12. The samples for analyses were excised from the LD muscle between the 7th thoracic and the last lumbar vertebra after carcass chilling at 2°C. The samples were then vacuum-packed and stored in a cold room at 2°C until to 10 days *post mortem*. Samples for the assessment of protein changes were taken 45 min and then 48, 96 and 240 h whilst those for the shear force evaluation – 48, 96 and 240 h *post mortem*.

Electrophoretic analysis and immunoblotting

Muscle tissue proteins were separated electrophoretically (SDS-PAGE) in the 15% polyacrilamide gel with the addition of 8M urea [Pospiech *et al.* 2001]. The separation was conducted in the vertical system using the SE 250 type apparatus of the HOEFER Scientific Instruments Company. The obtained gel was scanned with the Image Master® VCR apparatus of PHARMACIA Company and analysed using the IMAGE Master® 1D programme.

Selected muscle proteins were identified with the western blotting method according to Fritz and Greaser [1991]. Titin (anti-titin clone 9D10), desmin (anti-desmin clone DE-U-10) and Tn-T (anti-troponin T clone JLT-12) antibodies were employed.

Statistical

Statistical analysis of data was performed with the STATISTICA 8.0 programme using one-way and two-way analysis of variance. Arithmetic means, standard deviations (SD) and mean square (MS) of deviation from the analysis of variance were considered when comparing the individual traits. Significance of differences was verified using the LSD Fisher's test at the level of significance $P \leq 0.05$.

Results and discussion

The performed statistical analysis (the one-way analysis of variance as well as the analysis of mean squares of deviation (MS)) revealed that the first most frequent differentiating factor was the animals' breed (Tab. 1 and 2) which significantly affected protein changes in the muscle tissue. The next differentiating factor was the age of experimental animals, whereas the time of meat storage in the cold room turned out to be the least significant factor in this respect (Tab. 1 and 2).

With regard to the band of approximate molecular weight of 3700 kDa which corresponds to native titin (T1), its highest content (4.11%) was recorded in L bulls (Tab. 1). This value differed significantly from that found in H (2.86%) as well as in PHF (2.09%) bulls. Moreover, the muscle of PHF and H bulls contained the smallest amount of native titin. Beginning with hour 48 post-slaughter, the mean titin content decreased gradually to slightly less than 3% on day 10 of cold storage, which is typical for the progressing degradation process [Huff-Lonergan *et al.* 1995].

Table 1. Means and their standard deviations (SD) for individual proteins share in muscle (%) as related to breed (genotype) and age of bulls and duration of cold storage of their meat

Variation source			Protein molecular weight (kDa)						
			<42	42-200	105	200	<200-2400>	2400	3700
Total		mean	37.50	21.11	3.11	15.26	8.09	4.00	3.13
		SD	3.8	2.2	0.8	2.1	1.9	3.1	2.4
Breed	PF	mean	36.40^{a}	21.26^{b}	2.80^{a}	15.89^{b}	8.53^{b}	5.42^{b}	2.09^{a}
		SD	4.1	2.3	0.7	2.3	2.15	2.8	1.8
	H	mean	37.09^{ab}	20.20^{a}	2.87^{a}	15.83^{b}	8.81^{b}	4.98^{b}	2.86^{ab}
		SD	3.9	2.00	0.8	2.3	1.8	2.6	2.5
	L	mean	37.83^{bc}	21.55^{b}	3.44^{b}	14.87^{a}	7.75^{a}	2.64^{a}	4.11^{c}
		SD	3.2	2.3	0.8	1.8	1.6	3.1	2.5
	PR	mean	38.59^{c}	21.43^{b}	3.32^{b}	14.50^{a}	7.31^{a}	3.08^{a}	3.35^{bc}
		SD	3.8	1.9	0.8	1.9	1.6	3.1	2.4
Age	6 mo.	mean	35.61^{a}	21.55^{b}	2.99^{a}	16.29^{c}	8.52^{b}	4.43	3.20
		SD	3.2	2.4	0.9	2.2	2.0	3.2	2.4
	9 mo.	mean	37.58^{b}	20.90^{a}	2.95^{a}	15.42^{b}	8.10^{ab}	3.76	3.38
		SD	4.0	2.4	0.5	2.0	1.6	3.2	2.7
	12 mo	mean	39.21^{c}	20.89^{a}	3.40^{b}	14.13^{a}	7.69^{a}	3.83	2.81
		SD	3.4	1.6	0.9	1.7	1.9	2.9	2.2
Storage time	45’	mean	39.65^{b}	20.91	3.12	14.00^{a}	7.58^{a}	3.44	3.11
		SD	4.3	2.2	0.9	2.1	2.0	3.0	2.3
	48 h	mean	36.62^{a}	21.02	3.13	15.48^{b}	8.15^{ab}	3.92	3.27
		SD	3.0	2.1	0.8	1.8	1.7	3.3	2.6
	96 h	mean	37.06^{a}	21.34	3.14	15.51^{b}	8.22^{ab}	4.15	3.14
		SD	3.7	2.1	0.8	2.2	1.7	3.1	2.4
	240 h	mean	36.65^{a}	21.17	3.07	16.07^{b}	8.42^{b}	4.50	2.98
		SD	3.5	2.3	0.8	2.0	2.0	3.0	2.5

^{abc}Within columns for variation sources means bearing different superscripts are significantly different at P≤0.05.
PHF – Polish Holstein Friesian, H – Hereford, L – Limousine, PR – Polish Red.

Differences between the considered terms of analyses turned out to be not significant. Similar results, *i.e*. lack of effect of cold storage length within 12 days on meat protein degradation rate were reported by Kołczak *et al.* [2003b] when analysing washed myofibrillar proteins.

Considering the age factor, it was observed that the meat of 9-month old bulls was characterized by the highest (3.38%) content of native titin (Tab. 1) and differences between this group and the remaining two groups were non-significant. Similar results were obtained by Kołczak *et al.* [2003b] who reported that meat from 18-month old bulls contained more titin in comparison with 3-month and about 8-year old animals. The authors mentioned also found no significant differences between the compared animal groups.

The performed two-way analysis of variance confirmed the above-discussed relationships. Moreover, it indicated a simultaneous influence of breed and age of animals on the content of native titin during the first 45 min post-slaughter. This was

confirmed on the basis of the analysis of mean squares of deviations (MS) which, in the case of titin, showed a highly significant impact of breed on the course of the proteolytic process (Tab. 2).

Numerous authors [Maruyama 1986, Fritz and Greaser 1991, Matsuura *et al.* 1991] claimed that native titin is *post mortem* early degraded to the T2 band and fragments of 1200 kDa molecular weight. This phenomenon was confirmed by Wang *et al.* 1979, Boles *et al.* 1992, Huff-Lonergan *et al.* 1995 and Taylor *et al.* 1995] who found out that titin, beginning with day 3 *post mortem*, migrates as a double band in which the first one is referred to as T1 and the second – as T2 (2400 kDa). Fritz *et al.* [1993] and Huff-Lonergan *et al.* [1995], apart from T1 and T2 bands, distinguish a separate titin band migrating between T1 and T2 and name it T1-2 band.

One of the consequences of native titin (T1) degradation processes is an increase in products of its decomposition in the muscle tissue, *i.e.* proteins of 2400 kDa (T2) and of smaller molecular weight. Fritz and Greaser [1991], Huff-Lonergan *et al.* [1995] and Taylor *et al.* [1995] indicated that the amount of both products of native titin degradation (2400 kDa and 1200 kDa) increased during the first phase of *post mortem* ageing of meat.

Boyer-Berri and Greaser [1998] suggested that titin break-up close to line Z occurred after the first day of storage, whereas native titin degradation (T1) into a product of 2400 kDa (T2) was completed after three days.

Table 2. Mean squares of deviations (MS) from the analysis of variance for the share of bulls' muscle proteins in relation to age, breed and storage time of meat and interaction between variation sources

Variation source	Degree of freedom	Protein molecular weight (kDa)						
		<42	42-200	105	200	<200-2400>	2400	3700
Age – A	2	267.4***	8.8	5.142**	97.97***	15.45**	15.154	6.397
Storage time – B	3	123.6**	2.4	0.056	46.51***	7.69	11.405	0.778
Breed – C	3	61.0**	22.6**	6.300**	31.87**	30.27**	115.641***	41.772**
A × B	6	12.9	0.9	0.096	4.37	0.58	0.698	0.937
A × C	6	14.3	21.8**	1.479*	2.67	4.77	16.464	6.995
B × C	9	3.4	4.8	0.149	2.04	2.74	2.888	1.988
A × B × C	18	6.1	1.8	0.128	2.99	1.33	2.943	2.614
Error	187	11.2	4.3	0.650	2.98	3.40	9.141	6.088

*P≤0.05; **P≤0.01; ***P≤0.001.

Many authors [Bandman and Zdanis 1988, Huff-Lonergan *et al.* 1995, Taylor *et al.* 1995, Ho *et al.* 1996] claimed that a complete degradation of native titin (T1) into a band of 2400 kDa takes place during 3-12 days of cold storage, depending on the muscle type. However, Fritz and Greaser [1991] observed the titin band (T1) even

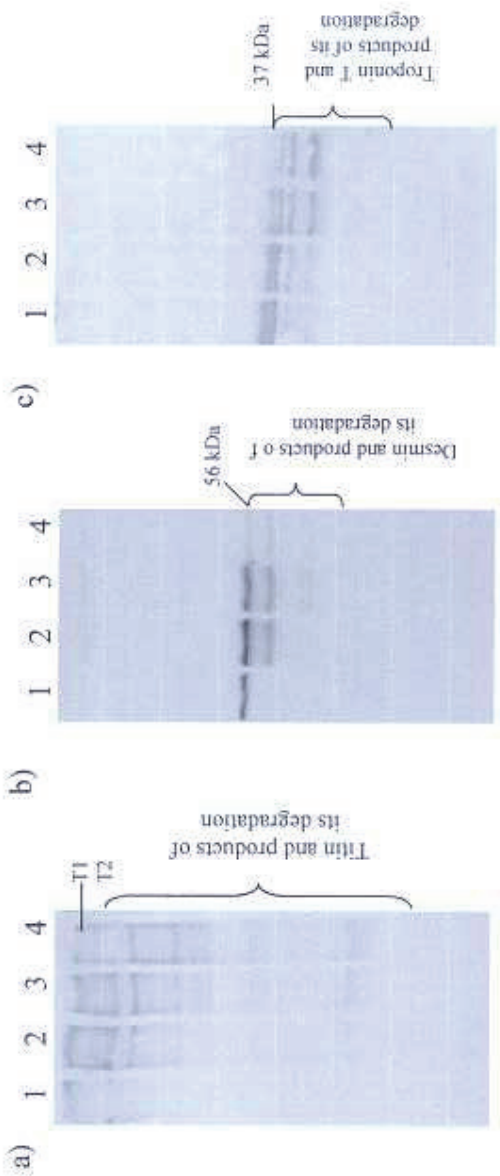

Fig. 1. Exemplary Western blotting electrophoregrams of selected proteins from muscle tissue (Limousine bull aged 9 months). a) – staining against titin (anti-titin clone 9D10); b – staining against desmin (anti-desmin clone DE-U-10); c – staining against Tn-T (anti-troponin T clone JLT-12). Lanes 1, 2, 3, 4 – samples obtained 45 min and 48, 96 and 240 h of cold storage, respectively.

after 16 days of cold storage. In the present study, the immunoblotting employing titin antibody confirmed its presence after 10 days of cold storage, although products of its degradation (T2) and of lower molecular weight were also identified (Fig. 1a).

The significantly higher content of 2400 kDa protein was found in PHF (5.42%) and H (4,98%) compared to L (2.64%) and PR (3.08%) bulls (Tab. 1). The greatest

content of this protein occurred in the muscle of the youngest animals (aged 6 months), although differences between the remaining two age groups were not found significant. Experiments conducted by Kołczak *et al.* [2003b] on washed myofibrillar proteins revealed that muscle tissue of both younger (aged 3 months) and older (aged 8 years) animals contained the same percentage of 205-2800 kDa proteins, while bulls at the age of 18 months showed a slightly higher content of proteins within the molecular weight in question.

Along with decline of titin (T1) increased (but not significantly) the content of 2400 kDa protein (T2) – from the lowest (3,44%) at 45 min post-slaughter to the highest (4.5 %) level on day 10 of cold storage. The significant influence of both breed and age of animals on protein changes 45 min and 48 and 240 h post-slaughter was revealed. The interaction of storage time with age was found significant only for PHF bulls (Tab. 2).

The same pattern of changes that occurred for proteins within the range from 200 to 2400 kDa was identified for the protein of 200 kDa (Tab. 1). During the 10-day period of cold storage, a continuous increase ($P \leq 0.05$) in the content of 200 kDa protein was observed – from 45 min post-slaughter up to the last date of analysis (14% and 16,07%, respectively – Table 1). These changes indicate that in the place of the band, which usually corresponds to myosin heavy chains (MHC), appeared also products of high-molecular protein degradation as indicated by the image obtained during immunoblotting with application of titin antibody (Fig. 1a). The highest content of 200 kDa protein was found in the muscle tissue collected from 6-month old bulls, the differences among all age groups occurring significant ($P \leq 0.05$). Significantly higher level of protein of this band was found in PHF and H than in L and PR bulls. It was revealed that in the case of 200 kDa protein, each of the three factors, *i.e*. breed, age and duration of meat cold storage affected significantly changes of 200 kDa protein, but no interactions were stated (Tab. 2).

Storage time did not affect changes in 105 kDa protein, which corresponds to α-actinin, the main element of the Z disc. This observation confirms suggestions of Taylor *et al.* [1995] that the protein in question does not undergo rapid degradation. Bechtel and Parrish [1983] and Hwan and Bandman [1989] suggest that α-actinin losses may occur after long-term storage (even 2-3 weeks) at 4°C and increase at higher temperature. In the present study the highest content of the 105 kDa protein ($P \leq 0.05$) was found in the tissue from 12-months old in relation to the younger bulls. With regard to breed, the higher content of protein of this band was recorded in L and PR than in PHF and H bulls. The breed, age and their interaction influenced significantly the changes of α-actinin (Tab. 2).

The process of degradation of proteins of the range 42-200 kDa proceeded similarly to the one described above, because many of these proteins constitute degradation products of high-molecular proteins and of 200 kDa. This is in accordance with results obtained by Sawdy *et al.* [2004] who found MHC fragments ranging from about 150 to about 154 kDa in loins after long ageing. Moreover, the appearance of these bands correlated (R^2=0.82) with meat tenderness on day 7 of cold storage.

Within the proteins of 42-200 kDa it is also possible to observe the desmin degradation changes (Fig. 1b). Beside several other cytoskeletal proteins, desmin constitutes the main costamere element. Taylor *et al.* [1995] revealed that over a half of desmin of the *semitendinosus* muscle was degraded between day 1 and 3 of cold storage, leading to a rapid increase in meat tenderness. In the present report the image obtained during immunoblotting with a desmin antibody showed a gradual decrease in desmin content, but not as dramatic as described by Taylor *et al.* [1995]. In the second and fourth day of cold storage the content of products of desmin degradation increased intensively, whereas the desmin band was conspicuous and disappeared only on day 10 of storage. Differences in desmin degradation intensity observed between these two trials result probably from the type of muscles as well as the cattle breed.

In the present investigation the highest content of 42-200 kDa proteins was identified at hour 96, while a drop was noted only on day 10 of cold storage (Tab. 1, Fig. 1b), differences between extreme values being negligible. The breed with the lowest level of 42- 200 kDa proteins was the H, differing significantly from remaining breeds. The youngest bulls showed the highest content of 42-200 kDa proteins, significantly ($P \leq 0.05$) differing from the remaining two age groups (Tab. 1). The analysis of the results from Table 2 revealed that only the breed significantly affected the course of proteolysis of proteins in question. A breed x animal age interaction was also observed.

The presence of proteins of below 42 kDa could be associated with the proteolysis of high-molecular weight proteins as well as those with lower molecular weights, such as Tn-T [McBride and Parrish 1977, Olson *et al.* 1977, Ho *et al.* 1994]. In addition, in the case of separation of muscle tissue proteins without washing (as was the case in the present trial) sarcoplasmic proteins may constitute a large group. Therefore, immunoblotting turns out to be a very suitable technique to assess changes in muscle proteins because they can be precisely defined. The evaluation of changes in proportions of proteins below 42 kDa revealed a significant difference between their contents directly post-slaughter (45') and at later terms of meat storage (Tab. 1). The performed immunoblotting with Tn-T antibody confirmed gradual degradation of Tn-T between the first 45 min and the last date of analysis (Fig. 1c). The highest content ($P \leq 0.05$) of low-molecular proteins was found in the tissue derived from PR compared to PHF and H bulls. Analysing the effect of age of bulls, differences between all groups ($P \leq 0.05$) in the level of proteins below 42 kDa were identified. Their highest content (Tab. 1) was observed in 12-month old animals, while in 6-month old – the lowest. Similar results were reported by Kołczak *et al.* [2003b] who found the highest content of Tn-T in the oldest cattle (aged 8 years). Statistical analysis showed that all the three factors considered in the present study, *i.e*. breed, age and length of meat cold storage influenced significantly changes concerning proteins of below 42 kDa. However, interactions between these factors were not found significant (Tab. 2).

The meat tenderness analysis revealed a gradual decline in shear force value, and consequently an increase of meat tenderness during the 10-day cold storage (Tab. 3),

Table 3. Means and their standard deviations (SD) for shear force of meat as related to breed and age of bulls across storage times of their meat (N/cm^2)

Variation source			Storage time		
			48 h	96 h	240 h
Total		mean	148.65^{c}	132.22^{b}	105.52^{a}
		SD	23.92	24.57	25.51
Breed	PHF	mean	139.81^{cde}	122.35^{bc}	104.81^{ab}
		SD	21.82	33.73	31.29
	H	mean	149.63^{ef}	134.51^{cde}	104.37^{a}
		SD	23.71	24.71	29.03
	L	mean	144.20^{def}	130.58^{cd}	100.44^{a}
		SD	25.80	21.89	24.89
	PR	mean	160.38^{f}	140.76^{de}	112.39^{ab}
		SD	21.29	13.02	15.66
Age	6 mo.	mean	149.26^{d}	129.66^{bc}	106.76^{a}
		SD	5.98	22.19	31.43
	9 mo.	mean	152.70^{d}	139.13b^{cd}	106.23^{a}
		SD	18.80	15.47	24.02
	12 mo.	mean	144.03^{cd}	127.73^{b}	103.62^{a}
		SD	26.64	32.53	21.64

^{abc}Within columns for variation sources means bearing different superscripts are significantly different at $P \leq 0.05$.
PHF – Polish Holstein Friesian, H – Hereford, L – Limousine, PR – Polish Red.

which is a typical phenomenon reported by other authors [Huff-Lonergan *et al.* 1996, Steen *et al*. 1997, Wheeler *et al.* 1999, Kołczak *et al.*, 2003b]. Differences in the shear force between the terms of analyses were more distinct than those caused by breed or age of bulls. The meat of PHF bulls exhibited the lowest shear force after 48 and 96 hours of storage and differed significantly from PR breed which was characterized by the highest shear force at all terms of analyses (Tab. 3). On the last day of cold storage (day 10) the mean shear force of meat samples of bulls of all breeds was similar. A lower shear force was determined for samples of 12-month old bulls, although after 48, 96 and 240 hours of storage significant differences between ages were not identified (Tab. 3).

According to Martin *et al.* [1971] and Ouali [1990] meat tenderness is affected by the origin and age of animals, their sex, breed, environmental conditions associated with the pre-slaughter stress, the slaughter itself as well as the time of meat ageing. The present investigation showed that changes in shear force in individual age groups were smaller than differences caused by the animals' genotype.

Protein changes usually reflect the progress in the process of meat tenderization [Koohmaraie 1994, Huff-Lonergan *et al*. 1995, Taylor *et al.* 1995, Koohmaraie and Geesink 2006]. The performed myofibrillar protein analysis (Tab. 1) demonstrated that the observed elevated tenderness of meat from the L and H bulls on day 10 of

cold storage was related to the lowest content of the titin T2 band as well as 200-2400 kDa proteins. This phenomenon was associated with the increase in bands of 200 kDa by 0.56 per cent points as well as decrease in low molecular weight proteins (below 42 kDa) content by 0.41 per cent points between day 4 and 10 of cold storage (Tab. 1). The low molecular protein fraction comprises Tn-T degradation products, an immunoblotting against Tn-T revealed an increased content of which. High relationships between shear force and Tn-T degradation process were reported by Huff-Lonergan *et al.* [1996], Steen *et al.* [1997] and Wheeler *et al.* [1999]. It was not, however, feasible to explain comprehensively whether Tn-T degradation was a basic part of the tenderization process or only an indicator of the *post mortem* proteolytic activity [Lametsch *et al.* 2003]. Also Taylor *et al.* [1995] suggested that Tn-T degradation to 30 kDa fragments was probably directly responsible for meat tenderization, but these observations fail to help solving the problem.

The impact of breed on the proteolytic process turned out to be of significance in the case of all the analysed meat proteins (Tab. 1 and 2). The performed one-way analysis of variance revealed similarities in the course of this process between the meat of PHF and H compared to the PR and L bulls (Tab. 1). Common traits within each of two pairs of these animals became apparent also during the analysis of the basic composition of their meat (Iwanowska *et al*., unpublished) as well as protein fractions of the centrifugal drip and meat physico-chemical properties (Magda *et al*., unpublished). It can be expected that the changes in degradation of centrifugal drip protein will provide some new information for the assessment of proteolysis in comparison with those resulting from meat tissue protein research since they are less numerous and it is easier to notice significant differences in their changes.

The results presented in this report can be summarized as follows.

- The factor which proved to have a decisive influence on the proteins content of muscle tissue was breed.
- The protein degradation processes taking place during the cold storage of meat from Polish Holstein-Friesian cattle are similar to those occurring in meat from Herefords whereas those occurring in meat of Polish Red cattle are closer to meat from Limousine breed despite the genotype differences.
- The intervals in animal age revealed differences in meat proteins degradation mainly between the extreme age groups, i.e. between month 6 and 12.
- Day 10 of meat cold storage was crucial for the degradation of most proteins. Apart from a decline in the titin (T1), desmin and Tn-T content, the increase in their degradation products became also apparent. At the same time, day 10 was the day of the highest meat tenderness.

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Zmiany zachodzące w białkach i kruchości młodej wołowiny zależnie od rasy i wieku ubijanych zwierząt

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

Analizowano zmiany następujące w białkach i kruchości młodej wołowiny przechowywanej w chłodni, zależnie od genotypu i wieku ubitych zwierząt. Badania objęły część piersiową i lędźwiową mięśnia najdłuższego grzbietu (LD) byczków czterech ras – holsztyno-fryzyjskiej odmiany czarno-białej (PHF), polskiej czerwonej (PR), hereford (H) i limousine (L), ubijanych w wieku 6, 9 i 12 miesięcy. Identyfikacji białek dokonano w 45 min i dalej w 48, 96 i 240 godzinie po uboju, za pomocą elektroforezy w żelu poliakrylamidowym z wykorzystaniem SDS (SDS-PAGE). W identyfikacji titiny, desminy i troponiny T (Tn-T) zastosowano przeciwciała tych białek i immunoblotting. Stwierdzono, że głównym czynnikiem wpływającym na udział badanych białek w tkance mięśniowej była rasa (a zatem genotyp) buhajków. Zaobserwowano podobieństwa w procesie degradacji białek między buhajkami PHF a H oraz między PR a L, mimo że reprezentują one różne genotypy. Uwzględniając oddziaływanie wieku, największe różnice w przemianach białek stwierdzono między mięsem uzyskanym od byczków 6- a 12-miesięcznych. Podczas składowania chłodniczego dzień 10 okazał się krytyczny pod względem degradacji większości białek. Zmniejszył się udział titiny, desminy i Tn-T, natomiast wzrósł udział produktów ich degradacji. Potwierdzeniem większych zmian degradacyjnych białek miofibryli była najlepsza kruchość mięsa w 10 dniu przechowywania.