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Effect of Aging Time on Meat Quality and Rate of Desmin and Dystrophin Degradation of Pale, Soft, Exudative (PSE) and Normal Turkey Breast Muscle

Dorota WOJTYSIAK and Magdalena GÓRSKA

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Original article

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The study evaluated the effect of aging time (30 min, 24 h, 48 h, and 72 h) on desmin and dystrophin degradation pattern, and meat quality of normal and PSE turkey breast meat. Characteristics of 30 normal and 24 PSE turkey breast meat samples, obtained from a commercial slaughter plant, were evaluated. Compared with normal meat, the PSE samples had significantly lower pH, greater L* and lower a* values in all the refrigerated storage periods under analysis. The cumulative drip loss for 30 min to 24 h, 30 min to 48 h, and 30 min to 72 h in PSE turkey breast samples was significantly greater than that from the normal group. Additionally, the intensity of intact desmin and dystrophin in PSE breast samples at 24 h, 48 h, and 72 h was significantly greater than that in the normal breast samples. Moreover, the rate of desmin degradation was associated with its localization in muscle fibres. In all muscle fibres, desmin was degraded more rapidly within rather than at the periphery of the fibres. Furthermore, the results indicate that the higher level of intact desmin and dystrophin in PSE breast samples could contribute to its higher drip loss during postmortem storage.

Key words: Turkey, dystrophin, meat quality, PSE, desmin.

Dorota WOJTYSIAK✉, Magdalena GÓRSKA, Agricultural University of Krakow, Department of Animal Anatomy, Institute of Veterinary Sciences, Al. Mickiewicza 24/28, 30-059 Kraków, Poland
E-mail: wojtysiakd@wp.pl
magdalena.gorska.ur@wp.pl

Poultry meat quality traits are affected by many factors such as species, breed, genetic line of birds, their housing conditions, preslaughter handling, and handling of meat during processing and storage. Some of the most important traits include water holding capacity, tenderness, and colour. Many decades of intensive selection for high weight and muscle gain have adversely affected poultry meat quality (BARBUT 1997; DRANSFIELD & SOŚNICKI 1999; BIANCHI *et al.* 2006; FRAQUEZA *et al.* 2006; ODA *et al.* 2009; STRASBURG & CHIANG 2009). The high rate of growth may cause some anatomo-histological and metabolic limits to be exceeded, which may make birds susceptible to various abnormalities in muscle tissue structure and function (GÓRSKA & WOJTYSIAK 2017, 2018; SOGLIA *et al.* 2018a). The biggest problem facing the poultry industry is PSE (pale, soft, exudative) meat, which causes substantial economic losses, lower productivity, and a decrease in raw material quality of meat (BARBUT *et al.* 2008; OWENS *et al.* 2009; CHAN *et al.* 2011; CARVALHO *et al.* 2014). This defect is attributed to a rapid rate of glycolysis and accumulation of considerable quantities of lactic acid, which with persistent high temperature leads to protein denaturation and reduces water binding and holding capacity (WARRISS & BROWN 1987). Low meat pH also favours myoglobin denaturation, thus contributing to the pale colour of meat. PSE meat is a concern not only for consumers, who usually purchase products based on their appearance, but

also a major concern for processors, because this type of meat in processing is inadequate not only because of its pale colour, but also due to its high drip loss, cooking loss, reduced juiciness, and poor emulsifying capacity (JOHNSTON *et al.* 2005). Moreover, exudative meat with lower water holding capacity is of limited use technologically (BARBUT *et al.* 2008).

It is generally accepted that during the process of meat formation, postmortem changes which occur in muscle tissue and determine subsequent production and culinary usefulness of the meat, are dependent, among others, upon the rate of cytoskeletal protein degradation (MORRISON *et al.* 1998; BEE *et al.* 2007; WOJTYSIAK *et al.* 2008; WOJTYSIAK & POŁTOWICZ 2015). When a cell loses homeostasis, the enzymes calpain and cathepsin are activated, which degrade cytoskeletal proteins, thus influencing meat quality (ILIAN *et al.* 2004; LAMETSCH *et al.* 2004; KEMP *et al.* 2009). MELODY *et al.* (2004) and ZHANG *et al.* (2011) suggested that the degradation of cytoskeletal proteins and protein oxidation can be involved in the regulation of drip loss during postmortem aging. In turn, YIN *et al.* (2014) indicated that PSE meat has a significant effect on the rate of cytoskeletal protein degradation during postmortem aging.

Therefore, the objective of this study was to determine the differences between normal and PSE (pale, soft, exudative) turkey breast meat in terms of pH, colour, drip loss, and desmin and dystrophin degradation during aging.

Material and Methods

Sample collection

The study was conducted on 54 turkey hens (BUT-9 line) aged 16 weeks with an average body weight of 9.5 kg. From one flock, boneless skinless whole turkey breast muscles were collected from the deboning lines of a local commercial processing plant from 238 turkey hens, after which, based on L* (lightness) colour parameter, 30 normal (L* values lower than 53) and 24 PSE (L* values greater than 53) breast meat samples were chosen according to the criteria of OWENS *et al.* (2000). The L* values were measured with a colourimeter on a total of 238 breast meats on the deboning line.

Meat quality analysis

To determine meat quality traits and desmin and dystrophin degradation, samples of turkey breast muscles were collected immediately after slaughter and divided so that all tests could be carried out on the slaughter day (30 min postmortem) and repeated at 24 h, 48 h, and 72 h postmortem. From each muscle, four large samples (about 150 g each) and two small samples (about 50 g each – to determine drip loss) were excised. The first large sample was used to determine the pH of meat, and to take muscle sections for all immunohistochemical analyses. The other three samples were vacuum packed and cold stored at 4°C to perform analogous analyses within the planned time frame of 24 h, 48 h, and 72 h postmortem. The samples for the determination of drip loss were cold stored in tightly sealed containers until the end of the experiment at 72 h postmortem.

Muscle pH was measured using a Matthäus (Germany) pH meter with a glass electrode standardized for pH 4.0 and 7.0 according to Polish Standard PN-77/a-82058 with automatic correction for muscle temperature at 30 min, 24 h, 48 h, and 72 h postmortem. The meat colour was assessed 30 min, 24 h, 48 h, and 72 h postmortem by the L* (lightness), a* (redness), and b* (yellowness) system (CIE, 1976) using a Minolta colourimeter (Chroma Meter CR-310, Minolta Camera C, Osaka, Japan). Colour was measured on the cranial, medial surface (bone side) in an area free of obvious colour defects. Drip loss was measured in duplicate samples. After thorough weighing (e=0.001g), samples were placed in sealed containers. After 24 h of storage samples were removed from their individual sealed containers and were towel dried, and weighed again. The chops were then placed in new sealed containers and stored for an additional hour. Following 48 h of storage, the samples were again towel dried and weighed. Similarly, the samples of meat were processed after 72 h of storage.

Muscle samples for immunohistochemical analysis were cut into 1 cm^3 pieces (parallel to the muscle fibers) and frozen in isopentane that was cooled using liquid nitrogen and stored at -80°C until subsequent analyses. Samples were mounted on a cryostat chuck with a few drops of tissue-freezing medium (Tissue-Tek; Sakura Finetek Europe, Zoeterwoude, The Netherlands). Transverse serial sections (10-µm thick) were cut at -20°C in a cryostat (Slee MEV, Germany). To determine desmin activity, frozen sections were stained as previously reported by WOJTYSIAK & POŁTOWICZ (2015). Briefly, sections were fixed with 4% formaldehyde, as paraformaldehyde (PFA), in 0.1M phosphate buffer (PB) (pH 7.4). Next sections were incubated for 30 min in 5% normal goat serum (NGS). They were then incubated overnight at 4°C with primary monoclonal antibody (NCL-DES-DERII, Novocastra, Leica, Germany)

at 1:100 dilution. After several washes in 0.01M sodium phosphate buffer (PBS) containing 0.05% Triton-X, sections were incubated overnight at 4°C with goat anti-mouse secondary antibodies conjugated to Alexa Fluor 555 (Molecular Probes) at 1: 250 dilution. After final washing, preparations were mounted in Vectashield medium (Vector Labs, Burlingame, CA) and examined with a Zeiss Axio Vision A.2 fluorescence microscope. In controls, there was immunostaining observed when primary antisera were omitted. In turn, to determine dystrophin activity, frozen serial sections were fixed with 4% formaldehyde, as paraformaldehyde (PFA), in 0.1M phosphate buffer (PB) (pH 7.4), and next after several washes in 0.01M sodium phosphate buffer (PBS), preparations were incubated in 10% H_2O_2 (10 min) to neutralize endogenous peroxidase. In the next stage, after washing in 0.01M sodium phosphate buffer (PBS), preparations were incubated with a primary mouse monoclonal antibody against dystrophin (NCL-DYS3, Novocastra, Leica, dilution 1:20), at room temperature for 1 h. The reaction was visualized by the NovoLink™ Polymer Detection System (Leica, Germany) according to the manufacturer's instruction. Finally, sections were dehydrated in a graded series of ethyl alcohol, cleared in xylene and mounted in DPX mounting medium (Fluka, Buchs, Switzerland). Sections were examined using a NIKON E600 light microscope.

Intact desmin and dystrophin were analysed using Western immunoblotting according to WOJTYSIAK & POŁTOWICZ (2015). To this end, whole muscle extracts were obtained by homogenizing muscle samples with 10 volumes of ice cold extraction buffer (pH 8.3) containing 50 mM Tris and 10 mM EDTA. The muscle homogenate was diluted 1:1 with protein denaturing buffer (4% SDS, 20% glycerol, 125 mM Tris, pH 6.8) and heated at 50°C for 20 min. Samples were centrifuged at 16000×g and protein concentration was determined using BCA assay (Sigma Chemical Co, St. Louis USA). Desmin was separated by SDS-PAGE on 10% separating acrylamide gel with 4% stacking acrylamide gel. In turn, dystrophin was separated by SDS-PAGE on 8% separating acrylamide gel with 4% stacking acrylamide gel. Gels for desmin and dystrophin were loaded with 60 μg of protein per lane. Relative molecular weights were determined using PageRulerTM Plus Prestained Protein Ladder (Thermo Fisher Scientific, Inc. Waltham, MA, USA). Discontinuous gels were run at 100V for 1.5 h. The gel was transferred to Immobilon-P transfer membrane (Millipore) overnight at 4°C and 30 mA in buffer containing 25 mM Tris, 193 mM glycine, and 15% methanol. After blocking with 10% non-fat dried milk in Tris-buffered saline pH 7.4 containing 0.05% Tween 20 for 1 h, the membrane was incubated at room temperature with primary monoclonal antibodies: NCL-DES-DERII (Novocastra, Leica, Germany, dilution 1:250) and NCL-DYS3 (Novocastra, Leica, dilution 1:20) for 2 h at room temperature. The membrane was washed three times (5 min) with TBST after each incubation. Bound primary antibody was visualized with goat anti-mouse alkaline phosphatase conjugate (Pierce Chemical Co., USA) diluted 1:2500, followed by detection of alkaline phosphate activity with bromo-chloroindolyl phosphate and nitroblue tetrazolium. The immunoblots were electronically scanned and the intensity of intact desmin and dystrophin was calculated using the densitometric method (ImageJ ver. 1.33U, National Institutes of Health, USA). Because the same amount of protein from each sample was loaded on SDS gels, the relative abundance of intact desmin and dystrophin at 30 min postmortem (as a reference standard to normalize the band intensities) of the normal and PSE samples was taken as 100% with respect to each muscle sample. The intact desmin and dystrophin contents at 24 h, 48 h, and 72 h postmortem were expressed as percentages of the 30 min postmortem samples. The obtained ratio was used for statistical analysis.

Statistical analysis

Differences among the PSE and normal breast samples and time of storage were analysed using 2-way ANOVA (General Linear Models procedure) in SAS software. The main effects of meat abnormality, storage time, and their interactions were evaluated and means separated using Tukey's test. No meat abnormality × the time of storage interaction was identified, so only main effects are reported and discussed. A probability of $P<0.05$ was considered statistically significant. The data were expressed as least squares means (LSM) ± standard error (SE).

Results and Discussion

Muscles form an important part of the carcass composition and are usually the main source of meat products for humans. The occurrence of meat defects has recently received much attention from many scientists and processors as PSE meat has been shown to have poor processing parameters and decreased consumer acceptance.

The results of the analysis of physicochemical parameters, including pH, lightness (L*), redness (a*), yellowness (b*) and drip loss of PSE and normal turkey breast muscle during 72 h storage are presented in Table 1.

Table 1

Postmortem changes in pH, colour (L*, a*, b*), and drip loss of PSE and normal turkey breast muscle. Values are expressed as least squares means (LSM) ± standard error (SE)

Item	Normal	PSE
pH		
30 min	6.09 ± 0.06^{Ax}	5.74 ± 0.04^{Bx}
24 h	5.95 ± 0.02^{ay}	5.63 ± 0.02^{by}
48 h	5.92 ± 0.04^{ay}	5.66 ± 0.04^{by}
72 h	5.89 ± 0.03^{ay}	5.64 ± 0.03^{by}
Colour		
L*		
30 min	47.96 ± 0.31^{ax}	56.42 ± 0.24^{bx}
24 h	49.54 ± 0.35^{ay}	58.43 ± 0.32^{by}
48 h	49.78 ± 0.52^{ay}	58.16 ± 0.65^{by}
72 h	50.82 ± 0.06^{ay}	59.04 ± 0.69^{by}
a*		
30 min	5.97 ± 0.21^{ax}	4.73 ± 0.15^{bx}
24 h	6.21 ± 0.08^{ax}	5.06 ± 0.12^{bx}
48 h	6.36 ± 0.16^{ax}	4.98 ± 0.07^{bx}
72 h	6.18 ± 0.09^{ax}	4.82 ± 0.08^{bx}
b*		
30 min	5.46 ± 0.23^{ax}	5.74 ± 0.28^{ax}
24 h	4.83 ± 0.18^{ax}	5.02 ± 0.34^{ax}
48 h	5.17 ± 0.22^{ax}	5.26 ± 0.19^{ax}
72 h	4.95 ± 0.14^{ax}	5.13 ± 0.21^{ax}
Drip loss [%]		
30 min - 24 h	0.74 ± 0.07^{Ax}	2.08 ± 0.05^{Bx}
30 min - 48 h	1.15 ± 0.06^{Ay}	2.83 ± 0.08^{By}
30 min - 72 h	1.49 ± 0.08^{Az}	3.25 ± 0.07^{Bz}

Values in rows with different superscript are significantly different: a, b ($P<0.05$); A, B ($P<0.01$).
Values in columns with different superscript are significantly different: x, y, z ($P<0.05$).

In poultry meat, the rate and extent of postmortem pH decline are important factors affecting meat quality (BERRI *et al.* 2001). A study on pigs (WARRIS & BROWN 1987) suggested that a decrease in pH at 1 h postmortem is the most important factor in developing PSE meat. On the other hand, FERNANDEZ *et al.* (1994) reported that ultimate pH (pHu) is a good indicator of development of PSE meat characteristics. Our analysis of the effect of the duration of refrigerated storage of meat on the pH value showed, as expected, a significant pH decline at 24 h compared to 30 min postmortem in both analysed groups of animals. We also noticed that at 30 min postmortem and in other periods of refrigerated storage of meat (24 h, 48 h, and 72 h postmortem) PSE turkey breast meat was characterized by significantly lower pH values compared to normal breast meat. The significant pH difference between normal and PSE breast meat is consistent with numerous studies on turkey and broiler meat (BARBUT 1993; OWENS *et al.* 2000; VAN LAACK *et al.* 2000; WOELFEL *et al.* 2002; PETRACCI *et al.* 2004; ÇELEN *et al.* 2016; KARUNANAYAKA *et al.* 2016). It is generally assumed that PSE develops when the rate of postmortem pH decline is high, leading to a low pH value while the muscle temperature is still high. This generally happens when postmortem glycolysis is dramatically accelerated (OWENS *et al.* 2000; FRAQUEZA *et al.* 2006; STRASBURG & CHIANG 2009).

Postmortem changes occuring during meat storage determine another quality parameter, namely colour. This is one of the main indicators of meat quality since any discoloration has a negative impact on customer choice. This parameter is also often used as one of the main characteristics of PSE meat (BARBUT 1993; KAUFFMAN *et al.* 1993; BARBUT 1998; WOELFEL *et al.* 2002; PETRACCI *et al.* 2004). OWENS *et al.* (2000) consider that measurement of the L* colour parameter is the best predictor of the PSE status, particularly in consideration of the fact that it is a nondestructive and noninvasive method compared with measurement of pH values. In our study, in the case of the L* a* b* colour parameters, in all the analysed meat storage periods, significantly higher L* values and significantly lower a* values were observed for PSE compared to normal turkey breast meat. On the other hand, there were no differences in b* values between fillet

types. These findings are consistent with previous studies on broiler chicken and turkey (VAN LAACK *et al.* 2000; BIANCHI *et al.* 2005; GARCIA *et al.* 2010). Different results were reported by ÇELEN *et al.* (2016), who compared normal and PSE turkey breast meat and noted that PSE meat had significantly higher a* and b* values compared with normal breast meat. Studies by SWATLAND (1993) indicated that the paleness of PSE meat is the result of denaturation of sarcoplasmic proteins, which increases light scattering in the muscle. Denaturation of protein can occur as a result of a rapid pH decline while the temperature of meat is still high (MITCHELL & HEFFRON 1982; OFFER 1991). Consistent with other studies, the colour of meat changes during its storage (PETRACCI & FLETCHER 2002; WOJTYSIAK *et al.* 2008; GARCIA *et al.* 2010). QIAO *et al.* (2001) and PETRACCI & FLETCHER (2002) reported that the L* value of breast muscles increased dramatically during the first hours of processing. In our study, L* values of both PSE and normal breast meat, increased from slaughter until 24 h postmortem, indicating that the meat became lighter during transformation of muscle into meat, including after the establishment of rigor mortis. At the same time, there were no significant differences in redness (a*) values and yellowness (b*) values. Meat lightness was possibly also related to its increased acidity (BARBUT 1993; CARVALHO *et al.* 2014; KARUNANAYALA *et al.* 2016). Thus, darker muscles had higher pH values and lighter muscles had lower pH values (FLETCHER 1999). Similar results were obtained by BOULIANNE & KING (1995), who showed a relationship between lightness of refrigerated breast fillets of chickens and the loss of heme pigments.

The most severe defect in the case of PSE meat is a high level of drip loss. It is obvious that total drip loss increases with storage of meat (ZHANG *et al.* 2006; BEE *et al.* 2007; WOJTYSIAK *et al.* 2008; WOJTYSIAK & POŁTOWICZ 2015). In our study, analogous results were noted for both PSE breast samples and normal breast samples during 72-h refrigerated storage of the meat. In addition, in all the meat storage periods under analysis, higher values of this parameter were found for PSE meat. Such a high drip loss during the first day after slaughter is associated with muscle fibre structure, in particular with myofibril orientation and sarcomere length. Myofibrils which make up the muscle fibres are built of two types of myofilaments: thin actin filaments and thick myosin filaments. According to BERTRAM *et al.* (2002), actin filaments contain more water than myosin filaments. Thus, during rigor mortis, which results from the sliding-in of thin filaments between thick filaments and leads to the shortening of sarcomeres, water loss from the cell is greater. In turn, significantly higher drip loss in PSE meat compared to normal meat, as suggested by OFFER (1991) and SANTOS *et al.* (1994), may be due to the fact that a rapid pH decline combined with high meat temperature cause denaturation of myosin. Myosin denaturation can be induced by insufficient pre-rigor chilling, which results in an increased drip loss (OFFER 1991; OFFER & KNIGHT 1988). Denaturation is speculated to occur in the head region and therefore results in a loss of ATPase activity. In addition, sarcomeres shrink laterally due to the formation of actomyosin and the drop of pH. OFFER & COUSINS (1992) observed a gradual lateral shrinkage of myofibres with a subsequent enlargement of space between fibres as well as fibre bundles from 2-48 h postmortem. During early postmortem a decrease in lattice spacing has also been shown by X-ray diffraction measurements due to the formation of actomyosin bonds between thick and thin filaments post, and pH decline (DIESBOURG *et al.* 1988). Hence, if the pH declines very fast postmortem, as in the case of PSE meat, the combination of low pH and high temperature will likely denature the myofibrillar proteins, shrinking the lattice transversally and thereby reducing the space for water to be held.

A major role in shaping postmortem meat quality parameters is played by cytoskeletal proteins. One of the key cytoskeletal proteins is desmin. It is also the main protein of intermediate filaments and contributes to maintaining cellular cohesion and integrity. Desmin connects myofibrils with each other and also with the cell membrane by contributing to the formation of costamere protein complexes. It has been established that degradation of cytoskeletal protein is a natural process that occurs during postmortem conversion of muscle into meat and continues during storage (HUFF-LONERGAN *et al.* 2010). However, earlier studies show that conditions that promote production of PSE pork are known to have negative effects on proteolysis of specific muscle proteins. YIN *et al.* (2014) showed in a study on pigs that intensity of intact desmin and troponin T2 in PSE pork was greater than that for RFN samples after 3 and 5 d of chilled storage. In turn, for integrin the same authors showed that more degradation of integrin was detected in PSE pork at 1 d compared to RFN samples.

The results of desmin degradation in PSE and normal breast meat during 72 h storage are presented in Fig. 1 and 2. Our immunohistochemical analysis and Western blot analysis showed that during storage of meat at 4°C the level of intact desmin gradually declined, and PSE breast meat was characterized by a significantly higher level of undegraded desmin measured at 24 h, 48 h and 72 h postmortem compared to normal breast meat. It is

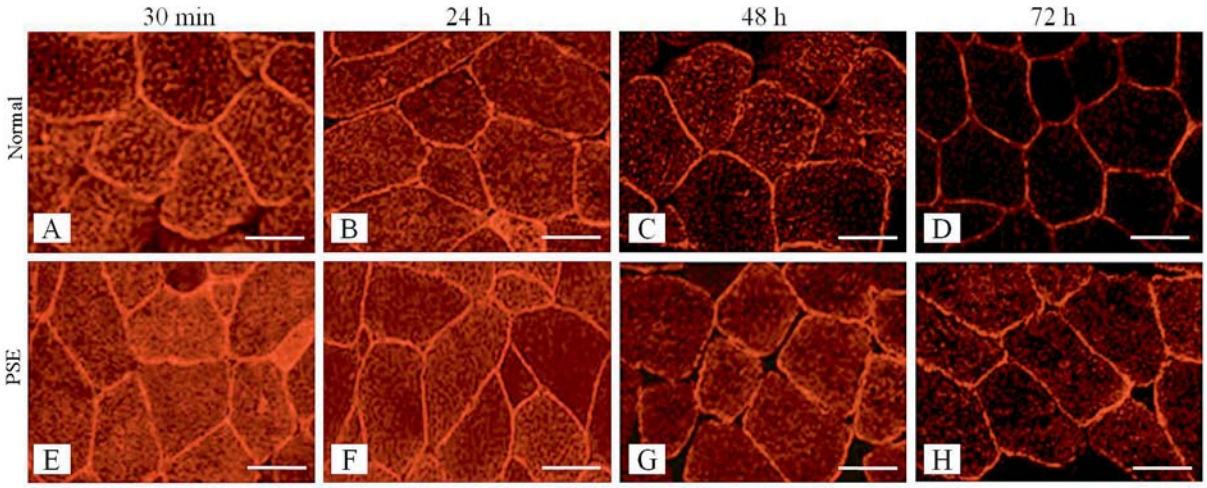

Fig. 1. Exemplary cross-section of normal (A, B, C, D) and PSE (E, F, G, H) turkey breast muscle: immunohistochemical detection of desmin (NCL-DES-DERII). Samples were stored for 30 min (A, E), 24 h (B, F), 48 h (C, G) and 72 h (D, H). Bar = 50 µm.

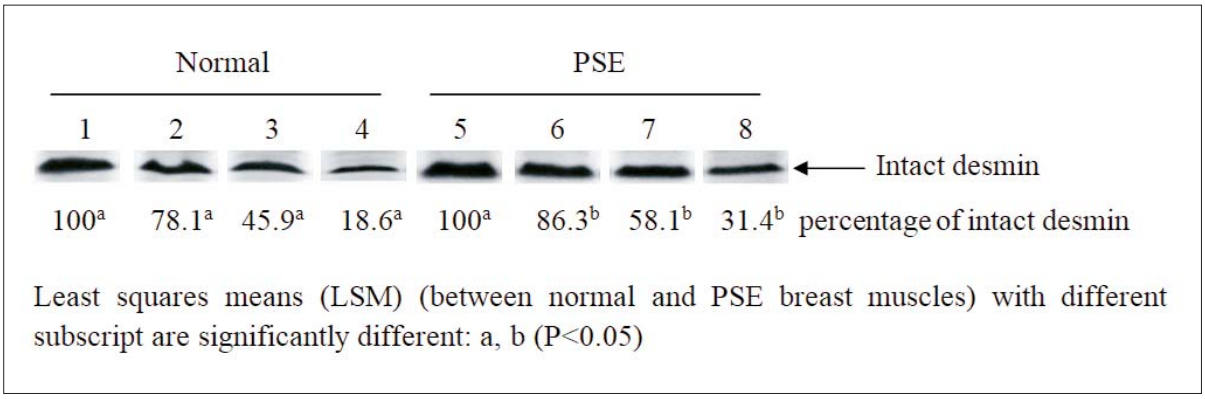

Fig. 2. Western blot analysis of desmin in normal and PSE turkey breast muscle. Samples were stored for 30 min (line 1, 5), 24 h (line 2, 6), 48 h (line 3, 7) and 72 h (line 4, 8).

worth noting, as demonstrated in an earlier study on pigs (WOJTYSIAK & POŁTOWICZ 2015), that the immunohistochemical reaction performed in our study and analysed under a confocal scanning microscope showed that the rate of desmin degradation also depends on its location on the muscle fibre. Accordingly, the earliest manifestations of desmin degradation were observed inside muscle fibres. The slowest degradation was observed for desmin located at the periphery of the muscle fibres.

Another important cytoskeletal protein contributing to costamere formation, is dystrophin. In muscle, by binding to the glycoprotein complex, dystrophin forms a major structural component of muscle fibres, responsible among others for the coupling of sarcolemma with actin filaments. In turn, by making specific links between the sacroplasmic cytoskeleton and extracellular matrix, it helps to maintain cell homeostasis (MINETTI *et al.* 1992).

The results of immunohistochemical analysis for dystrophin localization in PSE muscle fibres and normal breast meat during 72-h refrigerated storage of meat are presented in Fig. 3. Microscopic analysis showed that dystrophin in muscle fibres is localized on the inner cell membrane surface, and 30 min postmortem, this protein was found in all muscle fibres for both PSE and normal breast meat. At 24 h postmortem, in both analysed groups of animals, we observed muscle fibres in which the analysed protein was not present, and in PSE breast meat we noted only single myofibres with no dystrophin. In the successive meat storage periods (24 h, 48 h and 72 h postmortem), in both PSE and normal breast meat, we observed ongoing degradation of dystrophin in the muscle fibres, and importantly, in PSE breast meat the degradation of this protein was slower (involved a smaller number of muscle fibres) than in normal breast meat, where only trace amounts of dystrophin were observed in few muscle fibres 72 h postmor-

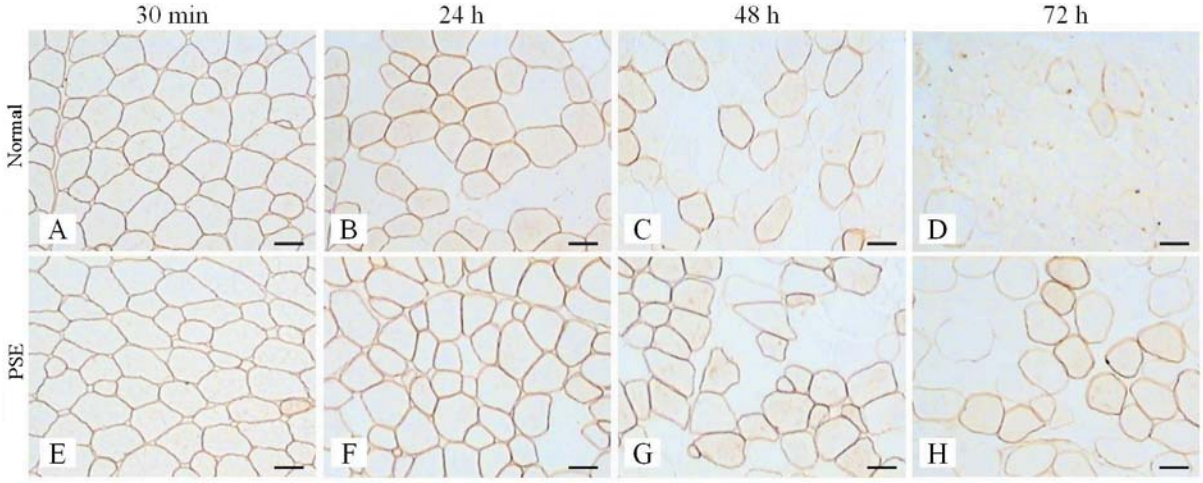

Fig. 3. Exemplary cross-section of normal (A, B, C, D) and PSE (E, F, G, H) turkey breast muscle: immunohistochemical detection of dystrophin (NCL-DYS3). Samples were stored for 30 min (A, E), 24 h (B, F), 48 h (C, G) and 72 h (D, H). Bar = 50 μm.

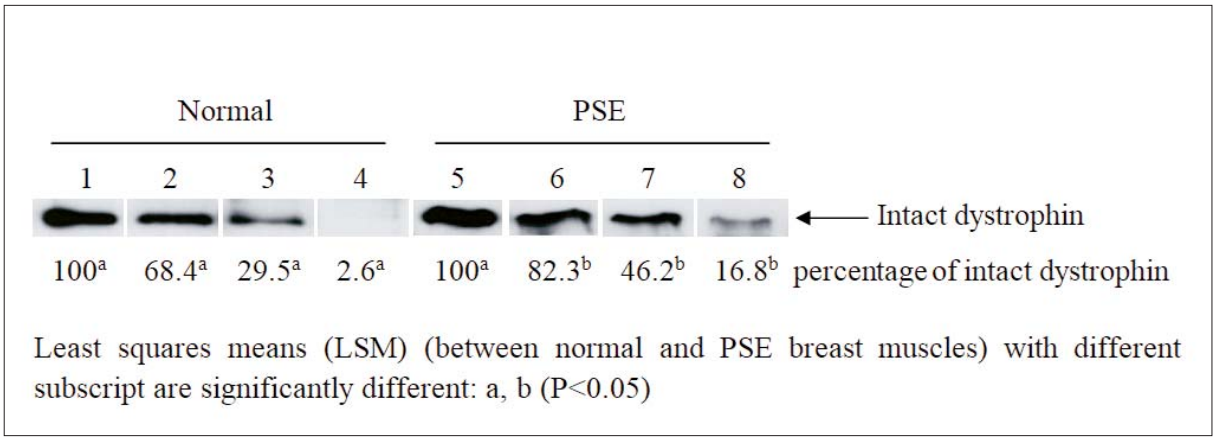

Fig. 4. Western blot analysis of dystrophin in normal and PSE turkey breast muscle. Samples were stored for 30 min (line 1, 5), 24 h (line 2, 6), 48 h (line 3, 7) and72 h (line 4, 8).

tem. These observations are also confirmed by our Western-blot analysis (Fig. 4), which demonstrated that dystrophin degradation during 72-h refrigerated storage of meat did not proceed evenly in both groups under analysis. Accordingly, at 24 h, 48 h and 72 h postmortem, the intensity of intact dystrophin in PSE breast meat was greater than that of normal breast meat. Moreover, in the case of normal breast meat at 72 h, dystrophin was degraded almost completely. The differences observed in our study in the rate of desmin and dystrophin degradation between PSE breast meat and normal breast meat may explain the differences observed in drip loss values between PSE and normal breast samples. As indicated by earlier studies, there is a strict relationship between the degradation rate of cytoskeletal proteins, including desmin, and meat parameters such as drip loss (WOJTYSIAK *et al.* 2008). ZHANG *et al*. (2006) and BEE *et al.* (2007) demonstrated that high levels of desmin degradation determine less drip loss during postmortem aging. In turn, a slower rate of desmin degradation has been proposed to result in the shrinkage of muscle cells to form gaps between muscle cells and muscle bundles which thus translate to a high drip loss during postmortem aging. HUFF-LONERGAN *et al.* (2002) showed that drip loss is dependent, on the one hand, on antemortem factors such as temperature and pH. On the other hand, it is associated with the presence of intracellular protein assemblies known as costameres, in which desmin and dystrophin analysed in our study form an important part. These structures make up a scaffold which ensures the attachment of myofibrils to the sarcolemma and thus they maintain normal muscle fibre structure. Accord-

ing to HUFF-LONERGAN & LONERGAN (2005), if costameric linkages remain intact during the conversion of muscle into meat, shrinkage of the myofibrils as the muscle goes into rigor would be transmitted to the entire cell via these proteinaceous linkages and would ultimately reduce the volume of the muscle cell itself. Thus, the rigor process could result in mobilization of water not only out of the myofibril, but also out of the extramyofibril spaces as the overall volume of the cell is constricted; this may explain the higher drip loss in PSE samples found in our study, where both desmin and dystrophin degraded more slowly compared to normal samples. It was reported that postmortem degradation of cytoskeletal protein is considerably influenced by the activity of proteolytic enzymes, namely calpains (KOOHMARAIE 1992). Calpains are cysteine proteases activated by Ca^{2+} ions. In avian skeletal muscle two ubiquitous calpain isoforms, μ- and μ/m-calpain, are present. LEE *et al.* (2008) reported that both calpains were activated and autolyzed in postmortem chicken muscles. CHANG & CHOU (2012) suggested that μ-calpain might play a dominant role in the postmortem proteolysis of skeletal muscle. In turn LEE *et al*. (2008) found that μ-calpain might be mobilized soon after slaughter, leading to very low activity 12 h postmortem. On the other hand, at longer postmortem times μ/m-calpain and its autolyzed form were identified as the dominant calpain from within the broiler breast meat (SOGLIA *et al*. 2018b). Autolysis of calpain is considered to be a hallmark for activation of calpain in postmortem muscle. It is therefore suggested that a greater proportion of the calpain catalytic subunit present as the 76 kDa autolysis product indicates that a greater proportion of calpain has been active. Studies on pigs demonstrated that PSE pork showed lower intensity of autolysed 76 kDa product and greater intensity of intact 80 kDa μ-calpain compared to RFN pork (YIN *et al.* 2014), which is due to the lower pH in PSE pork because μ-calpain is known to have less activity at lower pH *in vitro* (MADDOCK *et al.* 2005; CARLIN *et al.* 2006; HUFF-LONERGAN *et al.* 2010). Thus, such a high sensitivity of μ-calpains to meat acidity is most probably one of the principal causes of the differences observed in our study in the degradation rate of analysed proteins between PSE and normal breast samples: a reduced rate of desmin and dystrophin degradation was observed in PSE breast meat characterized by lower pH values in the analysed periods of meat refrigerated storage compared to normal breast meat.

In summary, we conclude that PSE turkey breast meat compared to normal breast meat is characterized by significantly lower pH, greater L* and lower a* values at both 30 min and 24 h, 48 h and 72 h postmortem. Moreover, PSE breast samples have a lower degree of protein (desmin and dystrophin) degradation compared to normal breast meat during postmortem ageing, which translates into a higher level of drip loss in the case of PSE meat.

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Author Contributions

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Conflict of Interest

The authors declare no conflict of interest.

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