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Jolanta Tomaszewska-Gras, Jacek Kijowski

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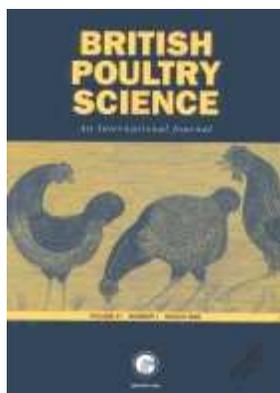
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Post mortem development of meat quality as related to changes in cytoskeletal proteins of chickens muscles

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2 ***Post mortem* development of meat quality as related to changes in cytoskeletal proteins**
3 **of chicken muscles**

4 J. TOMASZEWSKA-GRAS, F. J.G. SCHREURS¹ AND J. KIJOWSKI

5 *Faculty of Food Science and Nutrition , Poznan University of Life Sciences, Wojska Polskiego*
6 *31, 60-624 Poznan, Poland, ¹Institute for Animal Science and Health (ID-DLO), Lelystad,*
7 *The Netherlands*

8

Running title: Meat quality and cytoskeletal proteins

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22 **Correspondence to:** Dr J. Tomaszewska-Gras, Faculty of Food Science and Nutrition,
23 Poznan University of Life Sciences, Wojska Polskiego 31, 60-624 Poznan, Poland.

24 E-mail: gras@up.poznan.pl

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2
3 26 **Abstract** 1. A procedure was developed to separate high and medium molecular weight
4
5 27 myofibrillar proteins from chicken muscular tissue with a high resolution by flat bed sodium-
6
7 28 dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent detection
8
9 29 by either a general protein stain or Western blotting. These procedures were used to analyse
10
11 30 the degradation process of cytoskeletal proteins in chicken breast and leg muscles during meat
12
13 31 ageing.

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16
17 32 2. This study demonstrates the degradation of all the examined cytoskeletal proteins: titin,
18
19 33 nebulin and desmin as well as vinculin, a protein composing the costamere structure. All the
20
21 34 examined proteins were found to be degraded during ageing of chicken breast and leg
22
23 35 muscles.

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25
26
27 36 3. Degradation of titin, nebulin and desmin started at 3 h *post mortem* in breast muscle. Intact
28
29 37 titin and nebulin disappeared within 1 d. Intact desmin and vinculin were not detectable after
30
31 38 3 d *post mortem*. In leg muscle, the degradation process of all the examined proteins evolved
32
33 39 much more slowly than in breast chicken muscles.

34
35
36 40 4. The changes observed in shear force, myofibrillar fragmentation and cooking loss were
37
38 41 related to changes in cytoskeletal proteins and used to identify marker proteins or degradation
39
40 42 products for the purpose of monitoring the development of meat ageing. The ageing process
41
42 43 was faster in breast muscle than in leg muscle.

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44
45 44 5. Significant correlations were found between degradation processes of titin, nebulin, and
46
47 45 desmin and shear force, as well as myofibril fragmentation index of breast and leg muscles.
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50 INTRODUCTION

51
52
53 47 The development of meat quality during ageing is dependent on the *post mortem* loss of
54
55 48 longitudinal and lateral integrity of the muscular tissue, as a result of proteolytic degradation
56
57 49 of cytoskeletal proteins resulting in tenderisation of meat (Young *et al.*, 1980; Quali, 1992).
58
59 50 During *post mortem* ageing of meat, the major contractile proteins of muscle, *i.e.* actin and

1
2
3 51 myosin, together comprising approximately 80% of the myofibrillar mass, seem not to be
4
5 52 affected by proteolysis (Samejima and Wolfe, 1976; Yates *et al.*, 1983; Bandman and Zdanis,
6
7
8 53 1988). The most important structural components involved in meat ageing are considered to
9
10 54 be the cytoskeletal proteins, such as titin, nebulin and desmin (Koochmaraie *et al.*, 1984; Fritz
11
12 55 and Greaser, 1991; Robson, 1995) and vinculin (Taylor *et al.*, 1995). Titin, also called
13
14 56 connectin, is an extremely large monomeric protein found in striated muscle (Maruyama *et*
15
16 57 *al.*, 1977; Wang *et al.*, 1979) which forms a set of longitudinal filaments spanning from the
17
18 58 M-line region to the Z-line. Nebulin is considered to be a long molecule of molecular weight
19
20 59 of approx. 800 kDa which runs parallel to, and in close association with, the thin filaments.
21
22 60 Both titin and nebulin are largely responsible for the longitudinal integrity of the muscular
23
24 61 fibre. Desmin is a protein of approximately 53 kDa, present in intermediate filaments
25
26 62 connecting adjacent myofibrils at their Z-line levels (Robson, 1995) and one of the many
27
28 63 proteins composing the cell membrane skeleton, named costameres (Taylor *et al.*, 1995).
29
30 64 Desmin is considered to be the most important protein responsible for the lateral integrity of
31
32 65 the muscle fibre (Robson, 1995). Vinculin is one of the most important proteins composing
33
34 66 the costamere structure with a molecular weight of approx. 126 kDa and belongs to a chain of
35
36 67 proteins involved in forces of the myofibrils to the anchorites of the muscle (Taylor *et al.*,
37
38 68 1995).
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45 69 The kinetics of *post mortem* proteolytic degradation of these key cytoskeletal proteins is
46
47 70 of crucial importance for meat quality development. In the 1980s, most of the SDS-PAGE
48
49 71 studies were focused on the extremely large proteins, titin and nebulin, and their breakdown
50
51 72 (Lusby *et al.*, 1983; Paterson and Parrish, 1987). Depending on the molecular weight of
52
53 73 myofibrillar proteins, different percentages of total acrylamide and crosslinker have been used
54
55 74 throughout literature to produce gels of various porosities and with good separating
56
57 75 capabilities. For the extremely large myofibrillar proteins, such as titin and nebulin, with
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2
3 76 molecular weights of approximately 2800 and 800 kDa, respectively, highly porous
4
5 77 polyacrylamide gels have been utilised. These gels are extremely difficult to handle,
6
7
8 78 especially during Western blotting or staining, due to their fragile nature. The gels described
9
10 79 in literature for the separation of these high molecular weight proteins, without exception,
11
12 80 were of the 'vertical slab-gel type' (Studier, 1973; Porzio and Pearson, 1977; Paterson and
13
14 81 Parrish, 1987; Fritz *et al.*, 1989; Granzier and Wang, 1993).

15
16
17 82 Because of the limited sensitivity and separation of proteins by SDS-PAGE, it was
18
19 83 necessary to search for a new, more sensitive technique to detect proteins and their
20
21 84 degradation products, which are present in the muscle at very low concentrations.
22
23 85 Cytoskeletal proteins and some of their degradation products separated on these gels may be
24
25 86 detected very sensitively and specifically by Western blotting, first introduced by Burnette
26
27 87 (1981), however, chicken myofibrillar proteins are very difficult to separate (Chou *et al.*,
28
29 88 1994). Bandman and Zdanis (1988) successfully used Western blotting to study protein
30
31 89 breakdown in beef with antibodies to myosin and titin. This immunochemical method has also
32
33 90 been used in studies on titin and nebulin breakdown in bovine meat by Fritz and Greaser
34
35 91 (1991) and in studies on the breakdown of desmin in chicken muscle by Schreurs (1991).
36
37 92 Taylor *et al.* (1995) used Western blotting and electron microscopy to demonstrate that the
38
39 93 first change occurring in *post mortem* beef muscle was the disintegration of the N2-line and
40
41 94 costameres.
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48 95 Presently, little is known about the changes in cytoskeletal proteins of poultry meat, and
49
50 96 so the purpose of this study was to investigate the degradation of cytoskeletal proteins in
51
52 97 chicken muscle during *post mortem* ageing. Sample preparation, gel casting and Western
53
54 98 blotting were optimised for chicken muscle tissue. The utilised gels were ultra-thin, highly
55
56 99 porous polyacrylamide gels on a plastic backing film, and were used in a flat-bed gel-
57
58 100 electrophoresis set-up. This procedure resulted in very high-resolution electropherograms for
59
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3 101 high molecular weight proteins. The procedure was adapted to medium molecular weight
4
5 102 myofibrillar proteins, such as vinculin and desmin, using precast, commercially available
6
7 103 SDS-PAGE gels of 7.5 and 12.5 % total acrylamide, resulting in a comparably high
8
9 104 resolution.

10
11
12
13 105 In order to be able to relate changes observed in the cytoskeletal proteins to meat quality
14
15 106 characteristics, myofibrillar fragmentation as well as cooking loss and shear force were
16
17 107 measured in both breast and leg muscles.

18 19 20 108 MATERIALS AND METHODS

21
22 109 Water was always of Milli-Q (Millipore, The Netherlands) quality. Standard laboratory
23
24 110 chemicals were obtained from Merck and were of analytical grade, unless stated otherwise.
25
26 111 Precast ExcelGel SDS Homogeneous 7.5% (no. 80-1260-01) and gradient 8-18% gels,
27
28 112 ExcelGel SDS buffer strips (no. 17-1342-01) and Coomassie brilliant blue were obtained
29
30 113 from Pharmacia. Acrylamide/bisacrylamide and tetraethylenemethylene-diamine (TEMED)
31
32 114 were obtained from BDH. Reinforced nitro-cellulose membranes, pore size 0.45 μm , for
33
34 115 Western blotting were obtained from Schleicher and Schuell.

35 36 37 38 116 **Animals and sampling techniques**

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40
41 117 A total of 210 6-week-old Ross commercial broilers were obtained on two occasions from a
42
43 118 local slaughterhouse, transported to the Institute in typical commercial broiler crates, and
44
45 119 subsequently slaughtered at the in-house processing plant of the Institute for Animal Science
46
47 120 and Health (ID-DLO, Lelystad). Birds were stunned in a water bath stunner set at 100 V and
48
49 121 50 Hz for approximately 10 s, and the jugular veins severed with a rotating knife, but were
50
51 122 not scalded, plucked or eviscerated. After an approximate 3-min bleeding time, birds were
52
53 123 skinned and deboned. The initial (0 h) samples for electrophoresis were taken immediately
54
55 124 after excision of the muscles, frozen in liquid nitrogen, and stored at -80°C . Subsequently,
56
57 125 skinned breast and leg parts were wrapped in paper towels saturated with an antimicrobial

1
2
3 126 solution (10 mM sodium azide, 100 mg/kg chloramphenicol, 10 mg/kg riframycin) and kept
4
5 127 in a cold room at 4°C for sampling at 1, 3 and 6 h and further at 1, 2, 3, 5, and 7 d of *post*
6
7 128 *mortem* storage. Samples for SDS-PAGE and Western blotting, as well as for the
8
9 129 measurement of the myofibrillar fragmentation index (MFI), were taken repeatedly from the
10
11 130 same 9 carcasses at the above mentioned times *post mortem*.
12
13
14

15 131 The cooking loss and shear force measurements were carried out at 8 sample times on
16
17 132 12 different carcasses per sampling time (96 animals in total). The experiment described above
18
19 133 was repeated two times, which resulted in 210 animals being used in all the measurements: 18
20
21 134 carcasses (9 carcasses x 2 experiments) for SDS- PAGE, Western blotting and MFI, and 192
22
23 135 carcasses (12 carcasses x 8 periods x 2 experiments) for shear force, pH and cooking loss.
24
25
26

27 136 **Sample preparation**

28
29 137 Whole muscle samples (breast, *pectoralis major*, and thigh muscle, *biceps femoris*) for SDS-
30
31 138 PAGE and Western Blotting were taken (approximately 2 g) at the above times *post mortem*,
32
33 139 frozen in liquid nitrogen and stored at -80°C until analysis. On the day of analysis, samples
34
35 140 were again cooled in liquid nitrogen and subsequently pulverised using a liquid nitrogen
36
37 141 cooled mortar and pestle. The resulting powder was dissolved into a volume of sample buffer
38
39 142 according to Fritz and Greaser (1991), containing 25 mM Tris-HCl pH = 6.8, 8 M urea, 2 M
40
41 143 thiourea, 3% (w/v) SDS, 75 mM dithiothreitol (DTT) and bromophenol blue, to yield a
42
43 144 protein concentration of 1 mg/ml. Samples were heated for 20 min at 50°C and subsequently
44
45 145 cooled in tap water to room temperature and centrifuged at 12000 g for 5 min.
46
47
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49

50 146 **Polyacrylamide gel preparation**

51
52 147 Commercially available precast ExcelGels (12.5 x 260 x 0.5 mm) with homogeneous 7.5%
53
54 148 and a gradient of 8 to 18% total acrylamide were used. For the separation of high molecular
55
56 149 weight proteins, such as titin and nebulin, special gels of 3.2% total acrylamide
57
58 150 (bisacrylamide:acrylamide, 1:37) were prepared; 3.2% homogeneous polyacrylamide gels
59
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3 151 were cast in gel cassettes of 12.5 x 260 x 1.0 mm with sample wells of 5 x 5 x 1.0 mm (= 25
4
5 152 μ l), containing a GelBond PAG plastic backing film obtained from Pharmacia. The casting
6
7
8 153 solution was composed of 3.2 % acrylamide, 0.08 % N, N'-methylene-bisacrylamide, 375
9
10 154 mM Tris.HCl pH 8.8, 0.1% SDS, 0.03 mg/ml ammonium persulphate and 0.4% TEMED.
11
12 155 After polymerisation and disassembly of the gel cassettes, the gel was covalently bound to the
13
14 156 plastic backing film and could be easily removed from the gel casting moulds and positioned
15
16
17 157 on top of the cooling plate of the Multiphor II electrophoresis unit.

18 19 20 158 **Electrophoresis**

21
22 159 Flat bed SDS-PAGE was performed with a horizontal Pharmacia LKB Multiphor II System.
23
24 160 Muscle samples were loaded on 7.5% and 12.5 % gels (10 μ g of protein) and on 3.2% gel (20
25
26 161 μ g of protein). On the 7.5% and 12.5% gels, several aliquots of a mixture of Pharmacia low
27
28 162 molecular weight (LMW) and high molecular weight HMW-SDS (Pharmacia, 17-0615-01)
29
30 163 calibrators were applied to estimate the molecular weight of different bands. Onto gels used
31
32 164 for subsequent western blotting also a mixture of the above mentioned standards was applied
33
34 165 as well as samples prepared from muscle tissue that were frozen immediately after slaughter;
35
36 166 ExcelGel buffer strips (245 x 4.5 mm) were used as anodic and cathodic buffers. The strip
37
38 167 buffer system formed, together with the gel buffer, a discontinuous buffer system. High- and
39
40 168 low-molecular weight standards from Pharmacia were used to identify protein molecular
41
42 169 weights. Electrophoresis was performed at the constant current of 50 mA with the power
43
44 170 supply set at a maximum of 600 V and a maximum power of 30 W. The power supply was
45
46 171 equipped with a volt-hour (VH) integrator and electrophoresis was extended until 500 VH per
47
48 172 gel had been recorded.

49 50 51 173 **Fixation and staining**

52
53 174 Immediately after electrophoresis, gels were immersed in a fixing solution (40% ethanol, 10%
54
55 175 acetic acid) for 30 min. Subsequently, gels were incubated in the staining solution of 0.05%

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3 176 Coomassie brilliant blue R-250 (PhastGelBlueR, Pharmacia, no. 17-0518-01) in 25% ethanol,
4
5 177 8% acetic acid for 30 min, after which, gels were destained in the destaining solution (25%
6
7 178 ethanol, 8% acetic acid) until a clear background was obtained. Destained gels were soaked in
8
9 179 the preserving solution (10% acetic acid, 10% glycerol), and then dried covered with a clear
10
11 180 cellophane preserving film.
12

15 181 **Western blotting and detection**

16
17 182 Immediately after electrophoresis, as described above, gels were separated from the backing
18
19 183 film and transferred to a nitro-cellulose membrane, soaked in transfer buffer (25 mM Tris-192
20
21 184 mM glycine, pH = 8.3, 0.1% w/v SDS and 10% v/v methanol). Subsequently, proteins were
22
23 185 transferred from the gel onto the nitro-cellulose membrane by a semi-dry transfer method
24
25 186 using a constant voltage setting of 500 V for 120 min, and then blotted membranes were
26
27 187 incubated with blocking solution (Phosphate buffered saline (PBS) containing 0.1% Tween-
28
29 188 20 and 5% non-fat dry milk) for 30 min at 37°C to saturate protein binding places on nitro-
30
31 189 cellulose. Saturated membranes were later immersed in diluted primary antibody in
32
33 190 incubation buffer (PBS containing 0.1% Tween-20 and 0.1% non-fat dry milk) and incubated
34
35 191 overnight at 4°C in a moist environment. Antibodies and dilutions used were as follows:
36
37 192 Rabbit polyclonal antibody raised against titin at the Institute for Animal Science and Health
38
39 193 diluted 1:2 500
40
41 194 Rabbit polyclonal antibody to desmin, Sigma D-8281, diluted 1:400
42
43 195 Mouse monoclonal antibody to nebulin, Sigma N-9891, diluted 1:400
44
45 196 Mouse monoclonal antibody to vinculin, Sigma V-9131, diluted 1:200.
46
47
48 197 After overnight incubation, membranes were washed 3 times with incubation buffer to
49
50 198 remove excess antibody. Subsequently, membranes were incubated with a secondary antibody
51
52 199 alkaline phosphatase conjugate, either anti-rabbit (Boehringer Mannheim No. 1214-632;
53
54 200 diluted 1:2 000) or anti-mouse (Sigma A-3688; diluted 1:1 000), for 3 h at room temperature.
55
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1
2
3 201 The detection of alkaline phosphatase, and thus antigen-antibody complexes, was carried out
4
5 202 for 5 min using a 0.45 mg/ml solution of 5-bromo-4-chloro-3-indolyl phosphate/nitro blue
6
7
8 203 tetrazolium (Sigma No. B-5655) chromogenic substrate. The reaction was stopped by quickly
9
10 204 rinsing the membrane in water.

11 12 205 **Densitometry**

13
14
15 206 Gels and blots were scanned on a Hewlett Packard 6100C tabletop scanner with 400 dpi,
16
17 207 which is comparable to a resolution of 63.6 μm . Image scans were subsequently processed as
18
19 208 densitometer traces on a tabletop computer equipped with the Quantiscan programme from
20
21 209 Biosoft. From the analysis of lanes containing calibrator samples, a standard molecular weight
22
23 210 calibration curve was prepared. For the analysis of high molecular weight proteins, titin and
24
25 211 nebulin, a molecular weight calibration curve was prepared using bands appearing in the fresh
26
27 212 muscle sample lane. For this purpose, molecular weight of intact titin was set to 2800 kDa,
28
29 213 intact nebulin to 800 kDa, and myosin heavy chain to 212 kDa.

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33
34 214 Titin and nebulin contents were calculated by conversion to the known quantity of the
35
36 215 internal standard (β -galactosidase) introduced to the meat sample according to the method
37
38 216 published by Tomaszewska-Gras *et al.* (2002). A total of 18 gels were run (9 carcasses x 2
39
40 217 experiments). For gel densitometry analysis, a total of 13 (best stained, undisturbed) gels were
41
42 218 selected for each carcass from the two experiments.

43 44 45 219 **Myofibrillar fragmentation index (MFI)**

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47
48 220 Repeated MFI measurements were carried out according to Olson *et al.* (1976). In the MFI
49
50 221 method, absorbance was measured in a suspension of isolated myofibrils. This index defines
51
52 222 the degree of myofibril fragmentation to shorter fragments during meat ageing. This method
53
54 223 consists of the following stages: myofibril extraction, determination of myofibrillar protein
55
56 224 concentration and determination of the index. Samples (4 g) were weighed in two replications
57
58 225 from each cooled breast (*P. major*) and thigh muscles (three thigh muscles mixed together: *m.*

1
2
3 226 *quadriceps femoris*, *biceps femoris* and *m. semitendinosus*), and 40 ml MFI buffer chilled to
4
5 227 2°C were added to each sample. The composition of MFI buffer: 75 mM KCl, 10 mM
6
7 228 K₂HPO₄, 5 mM EDTA, 1 mM Na₃N, 1 mM PMSF, 1mM 1,4-disulfanybutane-2,3-diol, pH =
8
9 229 7.0. Samples were homogenised at a high rotational speed for 30 s and next centrifuged (1000
10
11 230 g for 15 min at 2°C). In order to remove sarcoplasmic proteins, the supernatant was decanted
12
13 231 and the remaining myofibril precipitate 40 ml MFI buffer added and myofibrils washed. After
14
15 232 myofibril precipitate was obtained, 20 ml MFI buffer were added. The suspension was filtered
16
17 233 through fine sieves (1 mm mesh size) in order to remove connective tissue. In such prepared
18
19 234 myofibril suspension, protein concentration was determined by the biuret method. A total of
20
21 235 0.25 ml was collected from the myofibril suspension in two replications and 0.75 ml MFI
22
23 236 buffer was added, to which 4 ml biuret reagent were added and the sample mixed. Samples
24
25 237 were placed in the dark for 30 min. Simultaneous with sample analyses, a standard curve was
26
27 238 prepared from bovine albumin (BSA) at concentrations of 0, 2.5, 5.0, 7.5 and 10 mg/ml.
28
29 239 Absorbance was read on a spectrophotometer at a wavelength of 540 nm. Based on the
30
31 240 standard curve and the size of absorbance, the protein concentration was determined in each
32
33 241 sample. Samples at a protein concentration of 0.5 mg/ml were prepared in two replications for
34
35 242 MFI measurements. After samples were mixed, their absorbance was measured at a
36
37 243 wavelength of 540 nm. The fragmentation index was calculated by multiplying the result by
38
39 244 200.

47 245 **pH measurement**

48 246 Changes in hydrogen ion concentration were measured directly in breast and thigh muscles
49
50 247 using a stiletto electrode of a CG - 837 pH-meter by Schott.

51 248 **Cooking loss and shear force**

52 249 Breast halves and legs designated for shear force measurements were weighed, vacuum sealed
53
54 250 and heated to a core temperature of 85°C in a boiling water bath and, immediately after

1
2
3 251 heating, brought back to 25°C in a water bath. Immediately before shear force measurement,
4
5 252 samples were removed from the sealing bag and blotted dry with a paper towel. Subsequently,
6
7
8 253 samples were re-weighed and weight differences before and after heating recorded as cooking
9
10 254 loss.

11 255 **Shear force measurements**

12
13
14
15 256 The cooked and chilled muscle samples were excised with a special scalpel, longitudinally to
16
17 257 the muscle fibres to reach sample dimensions: 1 x 1 x 5 cm. Breast muscle (*P. major*) and
18
19 258 three thigh muscles (*m. quadriceps femoris*, *biceps femoris*, *m. semitendinosus*) were taken for
20
21
22 259 shear force analysis. Measurements of shear force were carried out on an Adamel Lhomargy
23
24 260 instrument (Division Instruments S.A., Paris, France) with a single-knife Warner-Bratzler
25
26 261 attachment. Shear force values were expressed in N/cm².

27 262 **Statistical analysis**

28
29
30
31 263 All results obtained in the two experiments were statistically analysed and no effect of flock
32
33 264 found. Repeated measures ANOVA was carried out on the MFI data using the general linear
34
35 265 models module of the SPSS/Windows® statistical package. For cooking loss and shear force
36
37 266 measurements, a one-way ANOVA was used. Correlation coefficients of the relationships of
38
39 267 MFI, cooking loss and shear force with the optical density classification of the different
40
41 268 proteins bands on the Western blots was carried out with the Spearman rank correlation
42
43 269 module after the classification were converted to scores in the following manner: - = 0, ± = 1,
44
45 270 + = 2, ++ = 3, +++ = 4. Significant differences were identified at $P < 0.05$.

46 271 **RESULTS AND DISCUSSION**

47 272 **SDS-PAGE and Western blotting**

48
49
50
51 273 Results of SDS-PAGE and Western blotting analyses are shown in Figures 1, 2, 3 and 4.
52
53 274 Figure 1 shows results of SDS-PAGE for meat proteins on 3.2% (self-made) and Figure 2 on
54
55 275 7.5% (precast) total acrylamide gels. The lower gel concentration was used for the separation

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2
3 276 of proteins larger than 200 kDa in size. Bands of titin and nebulin from 7.5% gels were
4
5 277 determined quantitatively (Table 1) according to the method published by Tomaszewska-Gras
6
7
8 278 *et al.* (2002). Figures 3 and 4 show results of Western blots from 7.5% total acrylamide gels
9
10 279 with electropherograms of meat proteins developed with polyclonal anti-titin and monoclonal
11
12 280 anti-nebulin antibodies respectively, and from 12.5% total acrylamide gels with polyclonal
13
14
15 281 anti-desmin and monoclonal anti-vinculin antibodies respectively. Western blotting analysis
16
17 282 was performed to examine degradation products of proteins and their molecular weight. Table
18
19
20 283 2 shows results of semi-quantitative scoring of banding patterns that will be discussed below.

21
22 284 Titin degradation was analysed on the basis of SDS-PAGE with a 3.2% gel (Figure 1), a
23
24 285 7.5% gel (Figure 2) and Western blotting (Figure 3). The results from 3.2 SDS PAGE gel at
25
26
27 286 death showed that titin in breast and leg meat was visible as a closely-spaced doublet T1 and
28
29 287 T2; this is in agreement with other studies (Wang, 1982; Kimura *et al.*, 1984). With
30
31 288 increasing time *post mortem*, the amount of titin T1 decreased and the amount of titin T2
32
33
34 289 increased in all analysed samples. In breast meat, this phenomenon was most noticeable 6 h
35
36 290 *post mortem*, when the T2 band was much more intensive than in the 0-h sample. This
37
38 291 suggests that T2 was raised from the degradation of T1. The T1 to T2 conversion was
39
40
41 292 completed in all samples 24 h *post mortem*, when T1 was no longer detectable, whereas T2
42
43
44 293 remained present for 7 d. Leg muscle samples show a comparable conversion of T1 into T2.
45
46 294 Although the same amounts of protein were applied to gels in case of breast muscle samples,
47
48 295 leg muscle samples showed a larger number of smaller titin degradation products, appearing
49
50
51 296 as faint banding patterns between titin and nebulin, than was the case for breast muscle
52
53 297 samples.

Tables 1 & 2 and Figures 1, 2, 3 & 4 near here

54
55 298 The analysis of quantitative changes in titin and nebulin cytoskeletal proteins was
56
57 299 carried out by SDS-PAGE on 7.5% gel. For gel densitometry analysis, a total of 13 (best
58
59
60 300 stained, undisturbed) gels were selected for each carcass (breast and leg) from the two

1
2
3 301 experiments. The losses of intact titin and nebulin were determined quantitatively using a
4
5 302 known quantity of the internal standard, which had been introduced to each sample of meat
6
7
8 303 (Tomaszewska-Gras *et al.*, 1999, 2002). The analytical results are presented in Table 1. Titin
9
10 304 content decreased significantly in breast muscle after 1 d, whereas it was after 2 and 3 d of
11
12 305 refrigerated storage in thigh muscles. The observation of titin bands on gel revealed that the
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15 306 disappearance of the T1 form of titin, both in breast and thigh muscles, resulted in a
16
17 307 significant decrease of the titin quantity over the studied period. In case of nebulin, significant
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19 308 differences in its content were noted in breast muscle after 24 h and in thigh muscles after 48
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21 309 h of storage. It was also observed on gels that a significant decrease of nebulin content was
22
23 310 associated with the total degradation of its native form of 800 kDa to the form of 600 kDa.
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27 311 The Western blotting analysis with polyclonal antibodies to titin was used to detect
28
29 312 degradation products of titin. On the titin blot of breast muscle (Figure 3), 5 clearly visible
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31 313 titin degradation products were identified, referred to as Ta, Tb, Tc, Td and Te. Components
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33 314 Ta, Tc, Td and Te with molecular weights of approx. 1700, 1100, 900 and 700 kDa
34
35 315 respectively, already appeared early *post mortem* (at 3 h) as very faint bands. The relative
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37 316 amount of these components increased gradually until the second day *post mortem*, when the
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39 317 highest concentration was recorded except for Tc, of which the largest relative amounts were
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41 318 present during the first day after slaughter. The fifth component Tb, with a molecular weight
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43 319 of approx. 1400 kDa, appeared at 3 d *post mortem* just below titin T2. The T1 form was
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45 320 already degraded at that time. A more complex pattern of titin degradation products appeared
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47 321 in leg muscle, with some very distinct bands. At least 6 bands could be identified, designated
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49 322 Ta, Tb, Tc, Td, Te and Tf, with approximate molecular weights of 1900, 1400, 1100, 900, 700
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51 323 and 500 kDa respectively. The Ta, as well as the intact titin band, gradually decreased during
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53 324 the measuring period. All the lower molecular weight bands Tc, Td, Te, and Tf gradually
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55 325 increased until an intensity plateau was reached after 72 h. No disappearance of degradation
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3 326 bands was observed within 7 d *post mortem*, with the exception of the Tc band. It was faintly
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5 327 present from 0 h on and increasing in optical density during aging, but almost disappeared in
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8 328 the 7-d sample. The Tb component appeared in the 24-h sample and remained as a faint band
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10 329 up to 7 d *post mortem*. The presence of 1700 and 400 kDa degradation bands in chicken
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12 330 breast muscle had previously been reported (Kimura *et al.*, 1984), the first probably
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15 331 corresponding to the Ta band. Chou *et al.* (1994) identified a 1200 kDa component in chicken
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17 332 leg meat, but not in breast meat. In our study, corresponding 1100 kDa bands were identified
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19 333 in both muscle types. Tanabe *et al.* (1992) suggested that this component originates from T1
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21
22 334 and is composed of the Z-line side part of the T1 molecule.

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24 335 SDS-PAGE and Western blotting results prepared from 7.5% gels with monoclonal
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26 336 antibodies to nebulin, as shown in Figure 4, demonstrated that the degradation process of
27
28 337 nebulin in breast muscle started after 3 h as a small, diffuse smearing in the nebulin band and
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30 338 became more intense with the increasing storage time. The cleavage of nebulin into two bands
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32 339 was observed 6 h after slaughter; intact nebulin with a molecular weight of approximately 800
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34 340 kDa and an immunoreactive degradation product with a molecular weight of approximately
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36 341 500 kDa, designed Na. After 72 h of storage, a smaller degradation product, designed Nb,
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38 342 was found on blots with a molecular weight of approx. 400 kDa. After 5 and 7 d of storage,
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40 343 these two bands remained visible. In leg muscle, after 6 h only a small fraction of the original
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42 344 intact nebulin was left. After 24 h *post mortem*, most of the nebulin was degraded into a
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44 345 smaller cleavage product, designed Na, with a molecular weight of 500 kDa. Within 72 h *post*
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46 346 *mortem*, virtually all nebulin was degraded into smaller, no longer immunoreactive,
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48 347 fragments. Only small amounts of two fragments, the Na fragment and a new cleavage
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50 348 product designed Nb, with an apparent molecular weight of approximately 400 kDa, remained
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53 349 visible. On some blots the latter proteolytic fragment appeared as a closely spaced doublet.
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3 350 Based on blots, it may be concluded that the breakdown of nebulin was most prominent
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5 351 between 3 and 48 h, when intact nebulin was degraded and disappeared. This fact is
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8 352 consistent with previous reports, where the degradation of nebulin was analysed only by SDS-
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10 353 PAGE (Paxhia and Parrish, 1988; Chou *et al.*, 1994).

11
12 354 Figure 4 shows also the results of Western blotting with polyclonal antibodies to
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15 355 desmin. The results showed that in breast muscle, desmin degradation process started within 3
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17 356 h storage. Below the native band (53 kDa) of desmin, a faint band of approx. 47 kDa
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19 357 appeared, referred to as component Da. A new band was visible on the blot after 6 h ageing,
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21
22 358 designated Db, with a molecular weight of approx. 40 kDa. These two bands remained very
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24 359 strong until the second day. The most remarkable degradation of desmin in breast muscle
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26 360 occurred between 6 and 72 h *post mortem*. After this time, intact desmin disappeared
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29 361 completely and only three bands of products, *i.e.* Da (47 kDa), Db (40 kDa) and Db (34 kDa),
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31 362 remained until the seventh day of storage. In leg muscle, desmin degradation was much
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34 363 slower, with only very faint degradation visible after 24 h. At 48 h, the density of the native
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36 364 molecule started to become faint and clearly defined degradation products started to appear.
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38 365 The degradation pathway seems to be equal to that of the breast muscle, although much
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40
41 366 retarded. In other studies of desmin degradation by Western blotting analysis bovine meat was
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43 367 used (Hwan and Bandman, 1989; Taylor *et al.*, 1995). It has been established that the
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45 368 degradation of desmin in the bovine semimembranosus muscle, a fast-ageing muscle type,
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47 369 occurred between 24 and 72 h, while in bovine *biceps femoris*, a slow-ageing muscle type,
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49 370 desmin degradation was much slower.

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53 371 Degradation of vinculin was completed in breast muscle within 3 d *post mortem*, when
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55 372 intact vinculin was invisible on Western blots (Figure 4). In studies on vinculin degradation, it
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57 373 was difficult to establish the time when degradation started as a result of the use of a
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59 374 monoclonal antibody. The antibody is too specific to be able to pick up degradation products.

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3 375 In leg muscle, vinculin degradation was much slower, as is shown by the intact molecule still
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5 376 being present vaguely 7 d *post mortem*. A comparison of chicken muscle to the fast-ageing
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7 377 bovine semimembranosus muscle (Taylor *et al.*, 1995) showed that vinculin disappeared at
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9 378 the same time.

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12 379 In conclusion, it may be stated that on titin and desmin blots, the intact protein molecule
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14 380 and immunoreactive degradation products were visible, progressing in time *post mortem*.
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16 381 Monoclonal antibodies against nebulin also recognised some breakdown, as it was shown by
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18 382 the decrease in intact protein. The degradation pathway of nebulin generates some
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20 383 degradation products that are still recognised by the monoclonal antibody. The monoclonal
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22 384 anti-vinculin antibody was not capable of detecting degradation products of the native protein.
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24 385 This means that, in order to detect degradation products, the use of polyclonal antibodies is
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26 386 still preferred. Desmin and vinculin bands are not made visible on gels by Coomassie
27
28 387 staining. Desmin constitutes about 0.18% of skeletal muscle protein (Pearson and Young,
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30 388 1989). Vinculin has been calculated to comprise about 0.005% of breast muscle proteins
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32 389 (Maruyama, 1985). Both these proteins are easily visualised on Western blots.

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Table 3 near here

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41 391 **Myofibrillar fragmentation index (MFI) during *post mortem* storage**

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43 392 MFI is a measure of the average length of myofibrils and is significantly related to tenderness,
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45 393 *i.e.* with shorter myofibrils the index and meat tenderness values are higher (Taylor *et al.*,
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47 394 1995). Most studies on MFI have related this index at a specified time *post mortem* and most
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49 395 of them were conducted on beef. In this study we monitored changes in MFI in breast and leg
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51 396 chicken muscles during 7 d of storage. Table 3 shows the results of MFI measurement with
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53 397 the time *post mortem*. In breast muscle (*P. major*), MFI increases during *post mortem* ageing
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55 398 until it reaches plateau values after 24 h. In leg muscles (three muscles: *biceps femoris*,
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57 399 *quadriceps femoris*, *semitendinosus* considered as a whole), MFI increases gradually during

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3 400 the whole *post mortem* period that was investigated. Values recorded in leg muscle are
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5 401 approximately 0.5 the values observed in breast muscle. Differences in absolute MFI values
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7 402 indicate a scatter trend of breast muscle myofibrils different from the scatter trend for leg
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9 403 muscle myofibrils.

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12 404 The highest increase of MFI value in breast muscle was recorded 24 h after slaughter
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14 405 and reached approximately 100% of the initial value. In thigh muscles, MFI increased by
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16 406 approx. 60% of the initial value after 48 h of storage. This indicates much slower and less
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18 407 intensive myofibrillar degradation in leg than in breast muscles, which may be the effect of
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20 408 smaller amounts of proteolytic enzymes in thigh muscles. These results are consistent with the
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22 409 results of the SDS-PAGE and Western blotting experiments (Figures 1, 2, 3 and 4) and results
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24 410 reported in literature. In beef, MFI increases much more quickly in predominantly white
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26 411 muscles rather than in red muscles (Olson *et al.*, 1976). Similar results were observed by
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28 412 Taylor *et al.* (1995) on beef, where MFI index for the 'fast-aging' *semimenbranosus* muscle
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30 413 was higher than for the 'slow aging' *biceps femoris* muscle. Ultramicroscopic changes related
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32 414 to *post mortem* proteolytic degradation proceed much more quickly in chicken breast muscle
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34 415 than in leg muscle (Hay *et al.*, 1973).

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36 416 Earlier, it was generally believed that the increase in MFI after slaughter is caused by
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38 417 the weakening of the Z-disk, but electron micrographs (Taylor *et al.*, 1995) of myofibrils used
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40 418 in the MFI assay showed that Z-disks in these myofibrils were intact and that rupture of
41
42 419 myofibrils in the MFI assay occurred in the I-band (N2-line) and not at the Z-disk. This
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44 420 suggests that observed *post mortem* fragmentation of myofibrils is due to degradation of titin
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46 421 and nebulin, which are constituents of the N2-line (Funatsu *et al.*, 1990).

Table 4 near here

422 **Development of tenderness during *post mortem* storage of breast and leg muscles**

423 Table 4 shows the results of shear force measurements in different muscles with time *post*
424 *mortem*. Breast muscles (*P. major*) were tenderer than leg muscles throughout the

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3 425 investigation period. In breast muscle, the course of shear force changes is different from that
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5 426 in the investigated leg muscles because of differences in fibre type and different amounts of
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7 427 intramuscular connective tissue. It has previously been shown that in leg muscles (*biceps*
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9 428 *femoris*), thickness of perimysium is about two times higher than in breast muscles (Liu *et al.*,
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11 429 1996). Within 24 h *post mortem*, breast muscle exhibits its final shear force, however, the
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13 430 maximum shear force is reached at 6 h *post mortem*. The leg muscle shear force proceeds
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15 431 much more slowly, but its maximum is reached much earlier at 3 h *post mortem*. This
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17 432 indicates a faster rigor development in leg muscle than in breast muscle, but a slower *post*
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19 433 *mortem* ageing in the three leg muscles investigated. This is consistent with the results of the
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21 434 SDS-PAGE experiments (Figures 1, 2, 3 and 4) and the results reported in literature (Olson *et*
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23 435 *al.*, 1976; Hay *et al.*, 1973; Chou *et al.*, 1994).

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29 436 In studies on MFI, it was stated that in breast muscle MFI values reached the plateau at
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31 437 24 h *post mortem*, whereas the fragmentation process in leg muscles was finished at 48 h *post*
32
33 438 *mortem*. Based on the maximum toughness of breast muscle, 95% of all tenderisation
34
35 439 occurred 24 h *post mortem*. On the other hand, 81%, 89.5% and 86% of all tenderisation
36
37 440 occurred within 48 h of slaughter in three leg muscles (*biceps femoris*, *quadriceps femoris*,
38
39 441 *semitendinosus*).

Table 5 near here

442 **Cooking loss during *post mortem* storage**

443 Table 5 shows the results of cooking loss measurements with time *post mortem*. Cooking loss
444 observed in breast muscle gradually increases after slaughter. In leg muscle, cooking loss first
445 increased quickly, but at 6 h *post mortem*, it decreased again. The course of cooking loss
446 changes in leg muscle suggests a relationship with *rigor mortis* development. During the first
447 6 h after slaughter there is a drop in pH and an increase of shear force, when *rigor mortis*
448 occurs, the myofibrillar proteins are approaching the isoelectric point; this leads to a loss of
449 water holding capacity. However, the gradual increase of cooking loss in breast muscle

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3 450 throughout the investigation period suggests that there is a relationship between cooking loss
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5 451 and *post mortem* proteolytic breakdown of myofibrillar proteins. It is generally accepted that
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7 452 the cooking loss of meat is inversely correlated with pH, with a range of 5.0 – 6.5 being of
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9 practical importance; any alteration of pH has a large influence on the water holding capacity
10
11 453 of myofibrillar proteins (Pearson and Young, 1989). However, since the pH decline, as well
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13 454 as other *rigor mortis*-related factors, does not play a role in *post rigor* meat, the gradual
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15 455 increase of cooking loss has to be explained by some other mechanism. The degradation of
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17 456 the myofibrillar structure may play a role in the increase in cooking loss.
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22 458 **Relationship between the degradation process of cytoskeletal proteins and meat**
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24 459 **tenderness, MFI and cooking loss**

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27 460 In order to identify possible candidate marker fragments, semi-quantitative scores for relative
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29 461 intensity of different proteins and cleavage products on Western blots were converted to
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31 462 numerical scores as described in the Materials and Methods. Subsequently, the Spearman rank
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33 463 correlation coefficients were calculated for the relationships between these density scores and
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35 464 MFI, cooking loss and shear force measurements. For leg meat, shear force average was
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37 465 calculated for the three muscles tested. This was possible because shear forces were corrected
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39 466 for sample size. Table 6 shows the results of these calculations.

Table 6 near here

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43 467 In breast muscle, the degradation of titin as detected on Western blot did not correlate
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45 468 well with meat quality attributes. The trend for of fragment Tc, however, showed very high
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47 469 correlation coefficients with both cooking loss and shear force measurements. The decline of
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49 470 optical density of intact desmin as well as the course of optical density of the Dc fragments
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51 471 showed a high correlation with MFI, cooking loss and shear force, although the correlation of
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53 472 intact desmin with MFI was lower. The Db fragment showed a high correlation with MFI, but
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55 473 lower than the non-significant correlations with cooking loss and shear force measurements.
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57 474 The nebulin decline correlated highly with cooking loss and, to a lesser extent, with shear
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3 475 force measurements. Nebulin fragment Na was correlated only with MFI. There was only a
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5 476 5% correlation fragment of Nb with either cooking loss or shear force. The decrease of
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8 477 vinculin showed no significant correlation with any of the traits.
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10 478 In leg muscle tissue, titin fragments Ta, Tb, Td, Te and Tf showed significant
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12 479 correlations with MFI, cooking loss and shear force measurements. Only the decrease in the
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15 480 contents of intact titin and the Tc fragment bands showed low correlations. The decrease of
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17 481 intact desmin content and the increase in contents of fragments Db and Dc correlated
18
19 482 significantly with the course of changes in meat quality traits. The decrease of intact nebulin
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21 483 content and the increase in fragment Na content correlated significantly with meat quality
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23 484 parameters. The Nb fragment was only significantly correlated with shear force. The decrease
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25 485 in the level of intact vinculin molecules was significantly correlated with MFI and highly
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27 486 correlated with shear force.
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31 487 **Conclusions**

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34 488 It may be stated that the degradation of specific myofibrillar cytoskeletal proteins, as studied
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36 489 in the present work, follows the pattern of *post mortem* tenderisation and, as such, may be
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38 490 utilised as an indicator of meat ageing. Titin, nebulin and desmin degradations follow a course
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40 491 that is consistent with myofibrillar fragmentation and shear force. However, it has to be taken
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42 492 into account that processes involved in *rigor mortis* development, such as pH decrease, may
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44 493 interfere with these relationships. More research is needed to identify specific proteolytic
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46 494 fragments of titin, nebulin and desmin, which can serve as specific markers for the estimation
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48 495 of the degree of proteolysis. Once these components are identified, specific probes may be
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50 496 developed to quickly and easily monitor the course of meat ageing. We used Western blot
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52 497 analysis to identify some possible candidate protein fragments that might prove useful for this
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54 498 purpose. However, since Western blotting is a technique that may only be used to identify
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56 499 qualitative variations between different muscle samples it is unsuitable as a method for
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3 500 measuring quantitatively the course of *post mortem* proteolytic degradation of cytoskeletal
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5 501 proteins. Immunochemical techniques such as ELISA, however, are of a quantitative nature
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8 502 and future research will thus have to be carried out to develop similar methods, possibly in the
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10 503 form of 'dip-stick' methods or bio-sensors, using the above mentioned specific probes, to
11
12 504 monitor meat ageing in-line at the processing plant.

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5 598 cytoskeletal muscle. *Meat Science*, **5**: 41-55.
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Table 1. Changes in amounts of titin and nebulin determined by 7.5% SDS-PAGE, quantitative method with internal standards (breast, pectoralis major; leg, biceps femoris)

Time post mortem (h)	Titin content as percentage of total protein content		Nebulin content as percentage of total protein content	
	Breast n = 13	Leg n = 13	Breast n = 13	Leg n = 13
0	5.7±0.49 ^a	5.8±0.84 ^a	1.6±0.14 ^a	1.6±0.33 ^a
1	5.9±0.59 ^a	5.8±0.56 ^{ab}	1.7±0.17 ^a	1.4±0.30 ^a
3	5.8±0.51 ^a	5.5±0.63 ^{ab}	1.7±0.16 ^a	1.3±0.29 ^a
6	5.7±0.77 ^a	5.6±0.48 ^{ab}	1.6±0.24 ^a	1.4±0.40 ^a
24	4.6±0.62 ^b	5.5±0.48 ^{ab}	1.4±0.15 ^b	1.5±0.38 ^b
48	4.4±0.32 ^b	5.1±0.51 ^b	1.3±0.19 ^b	1.1±0.33 ^b
72	4.6±0.39 ^b	4.0±0.56 ^c	1.2±0.15 ^b	0.8±0.23 ^c
120	4.6±0.49 ^b	3.6±0.66 ^c	1.1±0.23 ^c	0.7±0.22 ^c
168	4.3±0.44 ^b	3.5±0.56 ^c	0.9±0.21 ^c	0.6±0.16 ^c

599 Group averages and standard deviations are shown.

600 Group averages not sharing a common superscript differ significantly within columns at $P \leq 0.05$.

601 n = number of samples from the same 13 carcasses.

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603 **Table 2.** Relative optical densities of bands on Western blots (number of blots n=3) in chicken breast (*P. major*) and leg (*biceps femoris*) muscles

	Fragment	Mw	Time post mortem (h)									
			0	1	3	6	24	48	72	120	168	
Breast (<i>P. major</i>)	Titin	2800	++	++	++	++	++	++	++	++	++	+
	Ta	1700	+	++	++	++	+++	+++	+++	++	++	++
	Tb	1400	-	-	-	-	-	-	±	++	++	++
	Tc	1100	+	++	++	++	+	±	-	-	-	-
	Td	900	+	+	++	++	++	+++	++	++	++	+
	Te	700	-	±	+	+	++	+++	++	+++	++	+
	Desmin	53	+++	+++	+++	+++	++	+	±	-	-	-
	Da	47	-	-	±	+	++	++	+	±	+	+
	Db	40	-	-	-	±	+	+	++	+	+	+
	Dc	30-37	-	-	-	-	±	±	+	+	+	+
	Nebulin	800	++	++	++	+	+	-	-	-	-	-
	Na	500	-	-	-	±	±	+	++	±	±	±
	Nb	400	-	-	-	-	-	-	±	+	+	+
	Vinculin	126	±	+	+	+	+	+	±	-	-	-
Leg (<i>biceps femoris</i>)	Titin	2800	+	++	++	++	+	+	+	+	+	±
	Ta	1700	++	++	++	++	++	+	+	+	+	+
	Tb	1400	-	-	-	-	±	+	+	+	+	+
	Tc	1100	±	±	±	±	±	+	+	+	±	±
	Td	900	-	±	±	±	+	+++	+++	+++	+++	+++
	Te	700	±	+	+	+	+	++	++	++	++	++
	Tf		-	-	-	±	±	+	+	+	+	+
	Desmin	53	+++	+++	+++	+++	+++	++	+	±	±	±
	Da	47		±	±	±	±	+	+	+	+	±
	Db	40	-	-	-	-	-	+	+	+	+	±
	Dc	30-37	-	-	-	-	-	±	+	+	+	+
	Nebulin	800	+++	++	++	+	+	-	-	-	-	-
	Na	500	-	-	-	-	±	+	±	+	±	±
	Nb	400	-	-	-	-	-	-	±	±	±	±
Vinculin	126	++	++	++	++	++	++	+	+	+	±	

604 **Table 3.** *Changes in myofibrillar fragmentation index (MFI) with time post mortem in breast*
 605 *(P. major) and leg muscles (considered as a whole)*

Time post mortem (h)	MFI	
	Breast (n = 18)	Leg (n = 18)
0	30.0 ± 7.9 ^a	18.0 ± 3.7 ^a
1	35.3 ± 9.0 ^b	20.5 ± 3.6 ^a
3	40.6 ± 10.7 ^c	23.3 ± 3.5 ^b
6	52.6 ± 9.1 ^d	25.1 ± 3.3 ^{bc}
24	62.8 ± 6.3 ^e	26.2 ± 4.5 ^{cd}
48	66.3 ± 4.8 ^e	30.7 ± 5.7 ^e
72	66.0 ± 5.0 ^e	30.1 ± 2.3 ^e
120	65.6 ± 6.8 ^e	28.7 ± 3.8 ^{de}
168	62.9 ± 9.4 ^e	33.5 ± 4.7 ^f

606 Group averages and standard deviations are shown.

607 Group averages not sharing a common superscript differ significantly within columns at P
 608 ≤ 0.05 .

609 n = number of muscle samples, measurements in 18 separate muscle samples, each made in
 610 duplicate.

611 **Table 4.** Shear force in *P. major*, *biceps femoris*, *quadriceps femoris* and *semitendinosus*
 612 *chicken muscles with time post mortem*

Time <i>post</i> <i>mortem</i> (h)	Shear force (N/cm ²)			
	Breast (<i>pectoralis</i> <i>major</i>) (n = 24)	Leg (<i>biceps femoris</i>) (n = 24)	Leg (<i>quadriceps</i> <i>femoris</i>) (n = 24)	Leg (<i>semitendinosus</i>) (n = 24)
0	35.8 ± 5.3 ^c	78.5 ± 11.1 ^c	116.9 ± 18.0 ^a	83.0 ± 19.0 ^b
3	46.6 ± 9.0 ^b	104.5 ± 18.9 ^a	92.6 ± 9.8 ^b	91.9 ± 19.4 ^a
6	93.3 ± 23.2 ^a	89.5 ± 13.5 ^b	95.7 ± 14.5 ^b	71.8 ± 19.2 ^c
24	17.9 ± 6.8 ^d	45.4 ± 6.8 ^d	56.9 ± 12.1 ^c	41.4 ± 8.6 ^d
48	15.8 ± 3.8 ^d	44.3 ± 6.1 ^d	39.1 ± 6.7 ^d	34.7 ± 6.7 ^{de}
72	16.3 ± 5.3 ^d	37.5 ± 5.7 ^e	35.4 ± 6.0 ^{de}	28.5 ± 6.0 ^{ef}
120	15.6 ± 3.0 ^d	34.3 ± 6.7 ^{ef}	32.7 ± 6.4 ^{de}	27.2 ± 3.8 ^{ef}
168	14.3 ± 3.3 ^d	30.0 ± 6.1 ^f	29.9 ± 7.1 ^e	24.6 ± 5.5 ^f

613 Group averages and standard deviations are shown

614 Group averages not sharing a common superscript differ significantly within columns at P
 615 ≤ 0.05 .

616 n = number of muscle samples, measurements in 24 separate muscle samples, each made in
 617 duplicate.

618 **Table 5.** *Changes in pH and cooking loss of breast and leg muscles during post mortem storage*
 619 *at 4°C*

Time <i>post</i> <i>mortem</i> (h)	pH		Cooking loss (%)	
	Breast n = 24	Leg n = 24	Breast n = 24	Leg n = 24
0	6.3±0.10 ^a	6.5±0.16 ^a	4.8±0.96 ^a	8.7±0.88 ^c
3	6.0±0.25 ^b	6.2±0.24 ^{bc}	6.2±1.14 ^b	9.8±1.73 ^d
6	5.6±0.25 ^c	6.1±0.13 ^c	6.9±0.81 ^{bc}	11.5±1.92 ^e
24	5.4±0.13 ^d	6.1±0.25 ^c	7.2±0.96 ^c	8.2±1.31 ^{bc}
48	5.6±0.19 ^{cd}	6.1±0.08 ^c	8.8±1.60 ^d	7.4±1.17 ^{ab}
72	5.5±0.15 ^{cd}	6.2±0.18 ^{bc}	8.8±1.57 ^d	6.9±1.36 ^a
120	5.6±0.14 ^c	6.2±0.18 ^{bc}	9.5±2.02 ^d	7.9±1.52 ^{bc}
168	5.8±0.19 ^e	6.3±0.10 ^{ab}	9.3±1.76 ^d	8.0±1.47 ^{bc}

620 Group averages and standard deviations are shown.

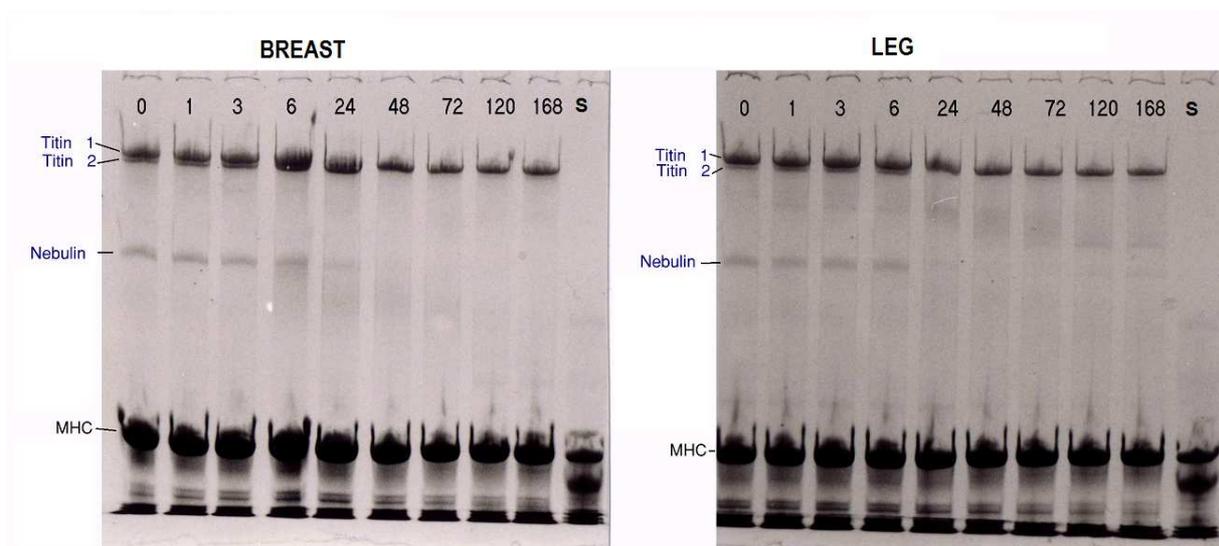
621 Group averages not sharing a common superscript differ significantly within columns at P
 622 ≤ 0.05 .

623 n = number of muscle samples, measurements in 24 separate muscle samples, each made in
 624 duplicate.

625 **Table 6.** Correlation coefficients determined for dependencies between changes in cytoskeletal
 626 proteins and qualitative parameters of poultry meat stored for 7 d at 4°C

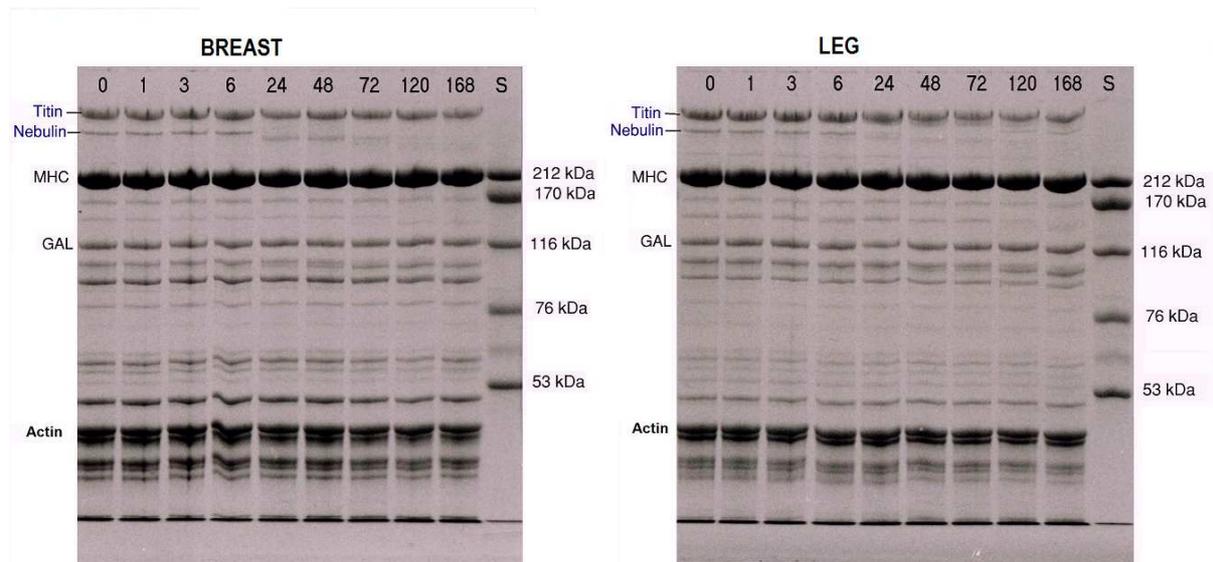
		Breast meat (<i>P. major</i>)			Leg meat				
		MFI	Cooking loss	Shear force			MFI	Cooking loss	Shear force
SDS-PAGE	Titin	-0.910**	-0.861**	0.909**	SDS-PAGE	Titin	-0.790*	0.581	0.880**
	Nebulin	-0.770*	-0.868**	0.780*		Nebulin	-0.877**	0.577	0.922**
Western blotting	Titin	-0.137	-0.415	0.577	Western blotting	Titin	-0.671*	0.619	0.770*
	Ta	0.597	0.177	0.013		Ta	-0.866**	0.873**	0.873**
	Tb	0.508	0.830*	-0.784*		Tb	-0.913**	-0.913**	-0.913**
	Tc	-0.728*	-0.845**	0.914**		Tc	0.548	-0.845**	-0.507
	Td	0.671*	0.180	0.069		Td	0.940**	-0.856**	-0.894**
	Te	0.898**	0.708*	-0.519		Te	0.913**	-0.809*	-0.861**
	Desmin	-0.796*	-0.963**	-0.945**		Tf	0.935**	-0.823*	-0.926**
	Da	0.713*	0.317	-0.210		Desmin	-0.817**	0.728*	0.932**
	Db	0.896**	0.777*	-0.664		Da	0.671*	-0.782*	-0.574
	Dc	0.802**	0.919**	-0.882**		Db	0.783*	-0.913**	-0.756*
	Nebulin	-0.935**	-0.931**	0.823*		Dc	0.820**	-0.809*	-0.913**
	Na	0.922**	0.617	-0.447		Nebulin	-0.949**	0.805*	0.907**
	Nb	0.508	0.830*	-0.784		Na	0.802**	-0.819*	-0.756*
Vinculin	-0.173	-0.384	0.546	Nb	0.639	-0.620	-0.845**		
				Vinculin	-0.677*	0.550	0.866**		

627 Correlations significant at * $P \leq 0.05$ level (2-tailed), ** $P \leq 0.01$ level (2-tailed).

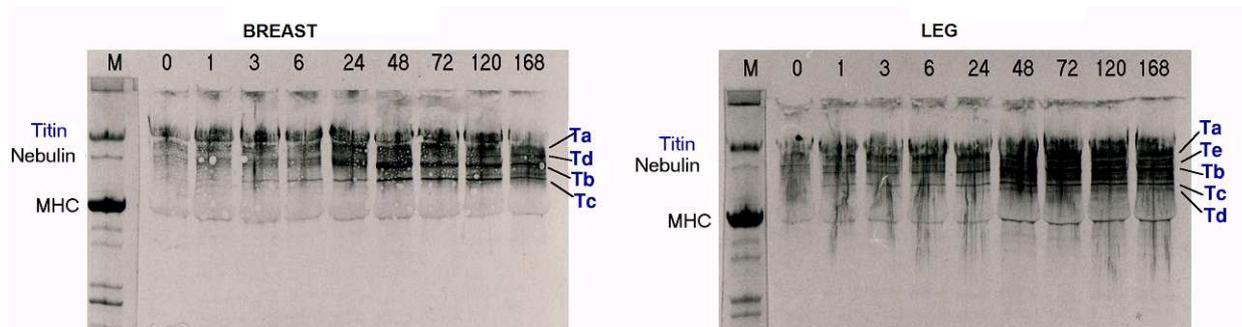


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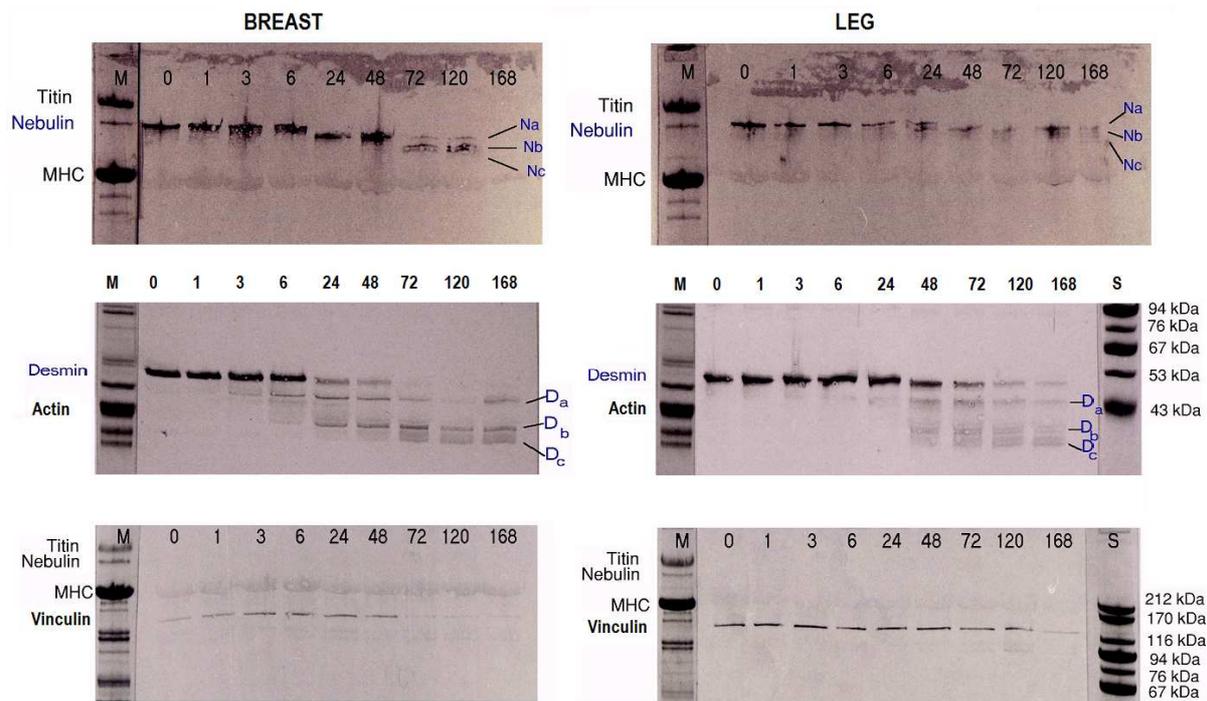
Figure 1. SDS-PAGE of 3.2% gel of chicken muscles (breast and leg) at 0, 1, 3, 6, 24, 48, 72, 120 and 168 h post mortem and stored at the temperature 4°C.



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 634 **Figure 2.** SDS-PAGE of 7.5% gel of chicken muscles samples (breast and leg) at 0, 1, 3, 6,
 635 24, 48, 72, 120 and 168 h post mortem when stored at 4° C with internal standard (GAL - β-
 636 galactosidase) S – marker proteins: myosin (212 kDa), α-macroglobulin (170kDa), β-
 637 galactosidase (116 kDa), transferrin (76 kDa) and glutamic dehydrogenase (53 kDa).



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 639 **Figure 3.** Western blotting of titin at 0, 1, 3, 6, 24, 48, 72, 120 and 168 h post mortem using
 640 polyclonal antibody to titin in breast and leg chicken muscles. M – SDS- PAGE of sample at
 641 '0' time.



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Figure 4. Western blotting of three cytoskeletal proteins at 0, 1, 3, 6, 24, 48, 72, 120 and 168 h post mortem using polyclonal antibody to desmin and monoclonal antibody to nebulin and vinculin in breast and leg chicken muscles. M – SDS- PAGE of sample at '0' time. S - SDS- PAGE of sample with molecular weight marker proteins.