



HAL
open science

ARE ONE EARLY MUSCLE PH AND TEMPERATURE MEASUREMENTS SUFFICIENT TO DETECT PSE BREAST MEAT IN TURKEYS?

Hervé Rémignon, Caroline Molette, Xavier Fernandez, Sunee Eadmusik

► **To cite this version:**

Hervé Rémignon, Caroline Molette, Xavier Fernandez, Sunee Eadmusik. ARE ONE EARLY MUSCLE PH AND TEMPERATURE MEASUREMENTS SUFFICIENT TO DETECT PSE BREAST MEAT IN TURKEYS?. *British Poultry Science*, 2011, 52 (02), pp.177-188. 10.1080/00071668.2011.554798 . hal-00686676

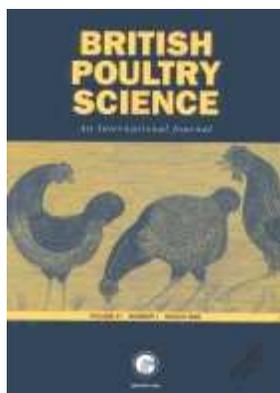
HAL Id: hal-00686676

<https://hal.science/hal-00686676>

Submitted on 11 Apr 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



ARE ONE EARLY MUSCLE PH AND TEMPERATURE MEASUREMENTS SUFFICIENT TO DETECT PSE BREAST MEAT IN TURKEYS?

Journal:	<i>British Poultry Science</i>
Manuscript ID:	CBPS-2009-095.R1
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	27-May-2010
Complete List of Authors:	Remignon, Herve; INPT, ENSAT-Animal Sciences MOLETTE, Caroline; INPT-ENSAT, Animal Sciences FERNANDEZ, Xavier; INRA, UMR tandem Eadmusik, Sunee; INPT-ENSAT, Animal Sciences
Keywords:	Meat, Turkeys, Protein

SCHOLARONE™
Manuscripts

CBPS-2009-095

Edited Lewis November 2010, MacLeod January 2011

1
2
3 1
4
5
6 2 **Are one early muscle pH and one early temperature measurement sufficient to detect**
7
8 3 **PSE breast meat in turkeys?**
9
10 4

11
12 5 S. EADMUSIK^{1,2}, C. MOLETTE^{1,2}, X. FERNANDEZ² AND H. RÉMIGNON^{1,2}
13
14 6

15
16
17 7 *¹Université de Toulouse, INPT, ENVT, UMR 1289 Tandem, Tissus Animaux, Nutrition,*
18 8 *Digestion, Ecosystème et Métabolisme; ENSAT, F-31326 Castanet-Tolosan Cedex, France,*
19
20 9 *and ²INRA, Auzeville, F-31326 Castanet-Tolosan, France*
21
22
23
24
25 10
26
27 11
28
29 12
30
31
32 13

33
34 14
35
36 15
37
38 16
39
40 17
41
42 18
43
44
45
46 19 **Correspondence to:** Prof. Hervé Rémignon, Université de Toulouse, INPT, ENVT, UMR
47
48 20 1289 Tandem, Tissus Animaux, Nutrition, Digestion, Ecosystème et Métabolisme; ENSAT,
49
50 21 F-31326 Castanet-Tolosan Cedex, France.
51
52 22 E-mail: remignon@ensat.fr
53
54 23

55
56 24 **Accepted for publication 13th October 2010**
57
58 25
59
60

1
2
3
4 26 **Abstract** 1. Within a large flock of turkey toms (2000 BUT9 conventionally reared and
5
6 27 slaughtered), early muscle pH measurements were randomly done to distinguish two groups
7
8 28 of birds presenting low (fast glycolysing, GR) or normal (normal glycolysing, GN) values.
9
10 29 2. Subsequently, ultimate pH values and meat quality parameters were also recorded. Meat
11
12 30 quality parameters from GR or GN samples differ more or less indicating more or less severe
13
14 31 PSE conditions. Proteins extracted from the samples at 20 min post mortem were quite similar
15
16 32 while they greatly differ at 24 h post mortem. Moreover, among the GR birds, a subgroup of
17
18 33 animals (called AB) presented SDS-PAGE profiles largely different from other GR or GN
19
20 34 birds.
21
22 35 3. All the subsequent analysis developed on meat quality parameters as well as for protein
23
24 36 extractabilities also differ between AB and other animals indicating that they must be
25
26 37 considered differently in term of PSE syndrome development.
27
28 38 4. Western blots against Myosin Heavy Chain and actin at 24 h post mortem indicate that
29
30 39 myofibrillar proteins alterations are different in AB and GR or GN samples.
31
32 40 5. At 20 min post mortem, glycogen content was lowest in AB samples while the glycolytic
33
34 41 potential was similar in all samples at the time of death. Measurements of PFK enzyme
35
36 42 specific activity did not indicate a different regulation of post mortem glycolysis in AB
37
38 43 samples.
39
40 44 6. Our results suggest that a unique pH measurement at 20 min post is insufficient to detect
41
42 45 animals more prone to developing a severe PSE syndrome in turkeys. In consequence, it is
43
44 46 suggested that a more precise evaluation of the kinetics of pH and temperature decrease has to
45
46 47 be conducted to understand the aetiology of meat quality parameters alterations in poultry.
47
48

48 INTRODUCTION

49 Since the beginning of the 1990's, the PSE (Pale, Soft and Exudative) syndrome has been
50
51 50 given a lot of attention from poultry scientists, as well as meat processors, because of its
52
53
54
55
56
57
58
59
60

1
2
3 51 adverse effects on poultry meat quality. In pigs, the genetic origin of the syndrome is well
4
5 52 established (*e.g.*, Barbut *et al.*, 2008), but it remains obscure in poultry (Rémignon *et al.*,
6
7 53 2007). Nevertheless, there is a large consensus in mammals, as well as in poultry, to consider
8
9
10 54 that the combination of a low pH and a high temperature in *post-mortem* muscle is at the
11
12 55 origin of protein alterations which lead to meat quality modifications (Alvarado and Sams,
13
14 56 2004). These alterations concern, to a greater or lesser extent, the colour of meat, its water-
15
16 57 holding capacity, its texture and also its ability to succeed in meat-product enhancements like
17
18 58 marination, curing-cooking processing or sausage elaboration (McKee and Sams, 1998;
19
20 59 Wynveen *et al.*, 1999; Alvarado and Sams, 2004).

21
22
23
24 60 Several facets of the production of live birds and/or management of carcasses have been
25
26 61 investigated, mainly in turkeys and chickens (for review, Barbut *et al.*, 2008), to describe the
27
28 62 factors which trigger the PSE syndrome. A lot of attention has also been given to the speed of
29
30 63 *post-mortem* glycolysis, which is thought to be more important than the animal itself (Choi *et*
31
32 64 *al.*, 2007; Gratacos-Cubarsi and Lametsch, 2008; Molette *et al.*, 2005; Hahn *et al.*, 2002;
33
34 65 Rathgeber *et al.*, 1999a). Indeed, an acceleration of muscle glycolysis is required to produce
35
36 66 low pH values at any time soon after slaughter, when the temperature of muscles is still high
37
38 67 (Petracci *et al.*, 2001). Several experiments have then been conducted to maintain poultry
39
40 68 muscle at high *post-mortem* temperatures and these have shown that such conditions lead to
41
42 69 poor meat quality if the rate of pH fall is not concomitantly reduced (Molette *et al.*, 2003;
43
44 70 Alvarado and Sams, 2002; Rathgeber *et al.*, 1999b). Studies have also been conducted to sort
45
46 71 animals according to their early muscle pH value and have demonstrated that fast glycolysing
47
48 72 muscles (*i.e.* with a low early muscle pH value) are generally associated with poor meat
49
50 73 qualities similar to those described when PSE or PSE-like syndrome develops (Warris and
51
52 74 Brown, 1987; Pietrzak *et al.*, 1997; Rathgeber *et al.*, 1999a; Wynveen *et al.*, 1999; James *et*
53
54 75 *al.*, 2006). Nevertheless, all these studies have observed the consequence of the presence of

1
2
3 76 both low pH and high temperature in poultry muscles at early times *post-mortem*, but no one
4
5
6 77 has yet proposed a clear origin of those conditions.
7

8 78 The measurement of early pH values cannot be generalised in processing plants because
9
10 79 it is destructive and not easy to perform. Soon after death, plasma membranes are still in their
11
12 80 native form and most of the water present in the muscle is retained in the myofibrillar
13
14 81 structure. Therefore, to have a good estimation of early pH values, it is absolutely necessary
15
16 82 to grind muscle samples in a buffer and to be able immediately to stop the anaerobic
17
18 83 glycolysis (iodoacetate is generally used as recommended by Jeacocke, 1977). On the
19
20 84 contrary, after the setup of *rigor mortis*, a large part of the water have been expelled from the
21
22 85 myofibrillar structure and released into the extracellular spaces after plasma membrane
23
24 86 disruptions. Thus, a direct measurement of proton concentration is possible with an adapted
25
26 87 (meat-type) pH electrode.
27
28
29
30

31 88 In consequence, although numerous studies are available on the variability of ultimate
32
33 89 pH (pHu) values within a given flock (Fernandez *et al.*, 2002; Hahn *et al.*, 2002; Fraqueza *et*
34
35 90 *al.*, 2006), few have paid attention to this variability in pH measurements made soon after the
36
37 91 birds have been slaughtered. Because muscle pH monitoring is not easily performed on a
38
39 92 slaughter line, authors generally report only one early pH value (recorded at 15 or 20 min
40
41 93 *post-mortem*) to define characteristics of fast or normal glycolysing muscles. Nevertheless,
42
43 94 we must consider that *post-mortem* glycolysis is a dynamic phenomenon which cannot be
44
45 95 described by a single measurement. The complexity of these measurements is increased if the
46
47 96 monitoring of internal muscle temperature has also to be taken into account.
48
49
50
51
52

53 97 Besides this, conclusions coming from the comparison of fast and normal glycolysing
54
55 98 muscles are not clear because some workers have reported alterations of sarcoplasmic
56
57 99 (Warner *et al.*, 1997; Joo *et al.*, 1999; Rathgeber *et al.*, 1999a) or myofibrillar proteins
58
59 100 (McKee and Sams, 1998; Rathgeber *et al.*, 1999a; Kuo and Chu, 2003). These protein

1
2
3 101 alterations may concern phosphorylase (Pietrzak *et al.*, 1997; Rathgeber *et al.*, 1999a,b;
4
5 102 Alvarado and Sams, 2004) or creatine kinase (Rathgeber *et al.*, 1999b) or, for example,
6
7 103 myokinase or triose phosphate isomerase and myosine (Rathgeber *et al.*, 1999b) or
8
9 104 cytoskeletal proteins such as titin (Warner *et al.*, 1997).

10
11
12
13 105 In the present study, we investigated whether all turkeys which exhibit a low early pH
14
15 106 value (20 min *post-mortem*) must be considered as a whole or if some of them could exhibit
16
17 107 different protein alterations which could make them more or less typical of the PSE
18
19 108 syndrome. To be sure that all the finally observed meat quality alterations were only due to
20
21 109 different glycolytic rates, all the birds were initially randomly selected from a commercial
22
23 110 flock with special attention being given to ultimate pH value (pHu) and glycogen reserves to
24
25 111 ensure that the amplitude of the decline in pH did not interact with the rate of pH fall.
26
27
28

29 112 MATERIALS AND METHODS

30 113 **Animals**

31
32
33
34 114 A flock of 2000 turkey toms (line BUT9, BUT Ltd., Chester, UK), was reared in a
35
36 115 commercial farm to 19 weeks of age (average live weight = 15 kg). All the birds were fasted
37
38 116 for 10 h before slaughter and then conventionally processed in a slaughter-house (Gastronome
39
40 117 SA, Moncoutant, France).

41 118 **Sampling**

42
43
44
45 119 At the end of the slaughtering process, the pH-values of 458 breast muscles (*Pectoralis major*,
46
47 120 Pm) were randomly measured (pH_{20min}) according to Jeacocke (1977). Two grams of breast
48
49 121 muscle were homogenised in 18 ml of 5 mM iodoacetate using an Ultra Turrax (IKA-
50
51 122 WERKE, Staufen, Germany). The pH_{20min} was then measured by using a portable pH-meter
52
53 123 combined with a glass electrode. The internal breast muscle temperature was also measured at
54
55 124 20 min *post-mortem* and 20 g of muscle were collected and immediately frozen in liquid
56
57 125 nitrogen.

1
2
3 126 Two groups were then sorted according to their $\text{pH}_{20\text{min}}$ values from all the measured
4
5 127 animals: the first one (Normal Glycolysis, GN, $n = 20$) was made up with animals showing
6
7
8 128 $\text{pH}_{20\text{min}}$ values similar to the mean value (6.21 ± 0.01) of the whole population, while the
9
10 129 second one (Rapid Glycolysis, GR, $n = 20$) was made up with birds presenting the lowest
11
12 130 $\text{pH}_{20\text{min}}$ values (5.80 ± 0.07). Following storage overnight at 4°C , pH_u was then directly
13
14
15 131 measured by inserting a glass pH-probe into the Pm muscle in GN and GR samples. At 24 h
16
17 132 *post-mortem*, other Pm samples were harvested as described above and kept at -80°C until
18
19 133 analysed.

22 134 **Meat quality measurements**

24 135 *Colour*

26 136 Meat colour was measured at 24 h *post-mortem* on the belly part of the breast meat with a
27
28 137 Minolta CR-300 chromameter (Osaka, Japan) The instrument was set to measure CIE L^* , a^* ,
29
30 138 b^* values using illuminant D and 65° standard observer.

34 139 *Water-holding capacity*

36 140 Drip loss was evaluated on scallops (around 100 g) of breast muscle collected 1 d *post-*
37
38 141 *mortem*. The scallops were then stored for 9 d at 4°C in a polystyrene tray covered with a
39
40 142 scattering film (air permeable). After final weighing, drip loss was expressed as a percentage
41
42 143 of the initial weight at 1 d (Honikel, 1998). After 9 d of storage, the scallops were weighed
43
44 144 (pre-thaw weight) and frozen at -20°C for 2 weeks. They were allowed to thaw overnight at
45
46 145 4°C and were then reweighed (precook weight). Thaw loss was expressed as a percentage of
47
48 146 the pre-thawing weight. The thawed meat was further used to evaluate cook loss and sausage
49
50 147 yield. The scallops were vacuum-packaged and cooked in an 80°C water bath for 15 min.
51
52 148 After cooking, samples were then cooled down to room temperature, rapidly wiped and
53
54 149 weighed. Cooking loss was expressed as a percentage of the precooking weight.

56 150 *Meat batter yield*

1
2
3 151 Sausages were produced following the method described by Shand *et al.* (1994). Thawed
4
5 152 meat (52.5g) was trimmed, chopped and mixed with iced water (21g, 0°C) and salt (1.5g) for
6
7
8 153 25 s using a Moulinex ILLICO (Lyon, France) blender. The meat batter was then transferred
9
10 154 to a plastic bag and vacuum packaged (residual pressure < 10mb) to remove air bubbles.
11
12 155 Twenty grams of meat batter were poured into a polypropylene tube and centrifuged at 700 g
13
14
15 156 at 4°C for 10 min. The tubes were then placed in a hot water bath until the internal
16
17 157 temperature of meat paste reached 80°C. After cooling down, the tubes were removed and the
18
19 158 sausages were weighed. The yield was expressed as a percentage of initial weight.

21 22 159 *Warner-Bratzler shear test*

23
24 160 The texture of cooked meat was evaluated according to Honikel (1998). A 1-cm-wide strip of
25
26 161 cooked meat was obtained by cutting parallel to the longitudinal axis of the scallop myofibres.
27
28 162 Shear force was measured using a MTS Synergie 200 apparatus (MTS, Eden Prairie, MN,
29
30 163 USA). The maximum force needed to shear the sample represented the meat tenderness and
31
32 164 was expressed in Newtons.

33 34 165 *Protein extractability*

35
36 166 Muscular proteins of Pm samples collected at 20 min and 24 h *post-mortem* were extracted
37
38 167 following the methods of Rathgeber *et al.* (1999a) and Boles *et al.* (1992). One gram of
39
40 168 sample was homogenised in 20 ml of a low ionic strength (LIS) buffer, containing 0.05 M
41
42 169 potassium phosphate buffer, 1 mM NaN₃ and 2 mM EDTA/pH 7.3 at 2°C for 10 sec. The
43
44 170 homogenised sample was then agitated for 30 min at 4°C and centrifuged at 17500 g at 2°C
45
46 171 for 15 min. Ten millilitres of the supernatant (LIS fraction) were collected at 2 cm above the
47
48 172 bottom of the tube while the remaining supernatant was discarded. A supplemental 20 ml LIS
49
50 173 buffer was added to resuspend the pellet which was then homogenised, agitated and
51
52 174 centrifuged as previously describe. The supernatant was discarded and the procedure was
53
54 175 repeated with high ionic strength (HIS) buffer, containing 0.55 M KCl, 0.05 M potassium

1
2
3 176 phosphate buffer, 1 mM NaN₃ and 2 mM EDTA/pH 7.3 at 2°C. Following centrifugation, 10
4
5 177 ml of supernatant (HIS fraction) was collected as described above and the excess supernatant
6
7
8 178 removed. The pellet was re-suspended in 40 ml of potassium phosphate buffer containing 75
9
10 179 mM KCl, 10 mM KH₂PO₄, 2 mM MgCl₂ and 2 mM EGTA/pH 7 and sonicated (Sonificateur,
11
12 180 Bioblock Scientific, Illkirch, France) for 1 min to solubilise the fraction (pellet fraction).
13
14
15 181 Protein content in LIS, HIS and pellet fractions were determined by the BCA protein assay kit
16
17 182 (PIERCE, Rockford, IL, USA). Results are expressed as a percentage of total proteins.
18
19

20 183 **Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis (SDS-PAGE)**

21
22 184 SDS-PAGE was performed in order to separate proteins in each buffer-extracted fraction from
23
24 185 samples collected at 20 min and 24 h *post-mortem*. Samples containing 2% (w/v) SDS, 5% β-
25
26 186 mercaptoethanol, 10% glycerol and 62 mM Tris-HCl, pH 6.8 in a ratio 1:1 v/v (Laemmli,
27
28 187 1970) were heated at 95°C for 5 min. SDS-PAGE was performed following the method
29
30 188 described by Laemmli (1970) using a Mini-Protean II electrophoresis unit or a Protean Xi
31
32 189 Unit (Bio-Rad Laboratories Inc., Hercules, CA, USA). Samples were loaded at 40 µg protein
33
34 190 per lane for LIS, HIS and pellet fractions, 5 and 10 µg for western blots of actin and Myosin
35
36 191 Heavy Chain (MHC), respectively. Resolving gel of 12% polyacrylamide was used for LIS
37
38 192 fraction and 8% polyacrylamide was applied for HIS and pellet fractions and western blots of
39
40 193 actin and MHC. Gels were run at 17 mA or 35 mA/gel (for Mini-Protean II and Protean Xi
41
42 194 Units, respectively), constant current, until the dye front reached the bottom of the gel. Gels
43
44 195 were stained overnight in Coomassie brilliant blue R-250 containing 5% (v/v) glacial acetic
45
46 196 acid, and 45% (v/v) ethanol. Gels were then destained twice in the same solution excluding
47
48 197 Coomassie blue.
49
50
51
52

53 198 **Western blots of Actin and Myosin Heavy Chain**

54
55 199 HIS fraction was used to perform western blots of actin and MHC. Following SDS-PAGE, gel
56
57 200 was steeped into transfer buffer containing 25 mM Tris, 192 mM glycine and 20% v/v
58
59
60

1
2
3 201 methanol for 20 min (Towbin *et al.*, 1979). Proteins were transferred to nitrocellulose
4
5 202 membrane (Hybond ECL, Amersham, Munich, Germany) at 30 V overnight using a transfer
6
7 203 cell (EBU 4000, CBS scientific, CA, USA). Membrane was blocked with milk buffer
8
9
10 204 containing 3% w/v milk powder in phosphate buffer saline (PBS, pH 7.5) at room temperature
11
12 205 (RT) for 1 h. Following blocking, membrane was probed for specific proteins using primary
13
14 206 antibody (mouse anti-actin, MAB 1501, Chemicon, CA, USA; mouse anti-myosin, F59,
15
16 DSHB, IA, USA) at RT for 2 h. The primary antibody to milk buffer ratio was 1:1000 and
17
18 207 1:25 for actin and MHC respectively. The membrane was then washed 3 times with PBS and
19
20 208 incubated in secondary antibody (goat anti-mouse IgG, AP 127, Chemicon, CA, USA) at RT
21
22 209 for 1.5 h. The secondary antibody to milk buffer ratio was 1:50,000 for actin and 1:25,000 for
23
24 210 MHC. Following this incubation, the membrane was washed 3 times and the
25
26 211 chemiluminescent substrate (Super Signal® West Pico, PIERCE) used to detect the reactivity
27
28 212 of the primary antibody with its antigen.
29
30
31
32
33

34 214 The Western blot was photographed by making a contact between the membrane and a
35
36 215 photo film (Amersham HyperfilmMP, GE Healthcare, Munich, Germany). The film was then
37
38 216 developed in 10% revelation solution (AL4, Kodak, NY, USA), distilled water and 10%
39
40 217 fixation solution (LX24, Kodak, NY, USA) for 1 min each, respectively. The amount of 2 and
41
42 218 5 µg proteins of chicken breast muscle were used as reference amounts for actin and MHC
43
44 219 respectively. Band intensity of samples was then measured by using Image Analysis
45
46 220 (ImageMaster 2D Platinum 6.0, GE Healthcare, NJ, USA). It was expressed as a percentage
47
48 221 of reference protein (actin and MHC of chicken breast muscle).
49
50
51
52

53 222 **Parameters of energetic metabolism**

54 223 *Assays of phosphofructokinase (PFK) and Cytochrome c Oxidase (COX) activities*

55 224 About 200 mg of Pm muscle was collected at 20 min *post-mortem* and homogenised with 4
56
57 225 ml of buffer containing 0.25 M sucrose, 2 mM EDTA and 10 mM Tris-HCL (pH 8.0) for 30 s
58
59
60

1
2
3 226 at 4°C. The enzyme extract was obtained from centrifugation at 10000 g for 5 min at 4°C. The
4
5 227 supernatant was collected and stored at 0°C. Activity of PFK was spectrophotometrically
6
7
8 228 measured in the presence of pyruvate kinase (PK) and lactate dehydrogenase (LDH) by using
9
10 229 a UVIKON 943 spectrometer (Kontron Instruments, Watford, UK) to follow a disappearance
11
12 230 of co-substrate (NADH) at 340 nm and 25°C for 2 min (Beutler, 1971). The reaction was
13
14
15 231 assayed by mixing 1 µl of enzyme extract with 1 ml of solution containing 0.07 M Tris-HCl
16
17 232 (pH 8), 1.4 mM MgCl₂, 4.5 mM KCL, 0.71 mM phosphophenol pyruvate, 0.65 mM fructose-
18
19 233 1,6- diphosphate, 0.4 mM NADH, 1 mM ATP, 1.8 mM fructose-6-phosphate, 0.08 U PK and
20
21 234 0.96 U LDH. The PFK activity was expressed as µmol of NADH disappeared per min and per
22
23
24 235 g of muscle (µmol/min/g).

25
26
27 236 The preparation of enzyme extract for COX activity was performed as described above
28
29 237 except that, after centrifugation, the supernatant was frozen and defrosted 4 times in liquid
30
31 238 nitrogen to break cell wall and to release the enzyme contained in the mitochondrial matrix.
32
33
34 239 The enzyme extract was kept at -20°C until used. Twenty µl of enzyme extract were mixed
35
36 240 with 1 ml of solution (pH 7.4) containing 90 µM Cytochrome c (reduced form) and 50 mM
37
38 241 potassium phosphate (Smith and Conrad, 1956). The activity was assayed at 550 nm and
39
40
41 242 25°C. The COX activity was expressed as micromoles of Cytochrome c (reduced form)
42
43 243 disappeared per min and per g of muscle .

244 *Glycogen, glucose, G-6-P, lactate and glycolytic potential determination*

245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000

245 Amounts of glycogen, glucose, glucose-6-phosphate (G-6-P) and lactate of Pm muscle
246 collected at 20 min and 24 h *post-mortem* were measured by enzymatic procedures according
247 to Dalrymple and Hamm (1973) and Bergmeyer *et al.* (1974). About 200 mg of Pm muscle
248 was homogenised in 10 ml of 0.5 M perchloric acid. Aliquots (0.5 ml) of homogenate were
249 taken for the enzymatic determination of glycogen and G-6-P after glycogen hydrolysis with
250 amyloglucosidase. The rest of the homogenate was centrifuged and the supernatant was used

1
2
3 251 for analysis of glucose, G-6-P and lactate. Glycogen content was calculated as the difference
4
5 252 between the results of the 2 sets. Lactate was determined in the supernatant resulting from the
6
7
8 253 centrifugation of the homogenate (20 min at 2500 g). Results were expressed in $\mu\text{mol/g}$ of
9
10 254 fresh tissue. Glycolytic potential was then calculated according to Monin and Sellier (1985) as
11
12 255 follows: $\text{GP} = 2[(\text{glycogen}) + (\text{glucose}) + (\text{glucose-6-phosphate})] + (\text{lactate})$. It was
13
14
15 256 expressed as μmol of lactate equivalent per g of fresh meat.

17 257 **Statistics**

18
19 258 Experimental data were analysed using the GLM-general factorial ANOVA procedure using
20
21
22 259 the SAS software system for Windows statistical package, version 9.1 (SAS Institute Inc.,
23
24 260 Cary, NC, USA). The GLM model was $Y_{ij} = \mu + G_i + e_{ij}$, where Y_{ij} = dependent observation; μ
25
26
27 261 = overall mean; G_i = effect of Group ($i = \text{AB, GR-AB and GN}$ in a model or GR and GN in
28
29 262 another one) and e_i = the residual error. Statistical significant effects were further analysed and
30
31 263 means compared using Student-Newman-Keuls multiple range test. Statistical significance
32
33
34 264 was determined at $P < 0.05$.

36 265 **RESULTS**

37
38 266 SDS-Page profiles are reported in Figure 1. For the different proteins extracted at 20 min
39
40
41 267 *post-mortem*, gel patterns were very similar indicating that samples from the different groups
42
43 268 of birds probably contained the same proteins. In contrast, at 24 h *post-mortem*, if
44
45 269 sarcoplasmic proteins (LIS buffer extracted) presented the same profile, noticeable differences
46
47
48 270 were present for HIS buffer and pellet protein extracts. Those differences concerned mainly
49
50 271 two bands, located at 200 and 45 kDa respectively, which were identified by mass
51
52 272 spectrometry analysis (data not shown) as myosin heavy chain (MHC) and actin respectively.
53
54 273 Whereas all the GN samples presented similar SDS-Page profiles, GR samples could easily be
55
56
57 274 divided in two groups. The GR-AB (Fast Glycolysing animals not ABnormals) samples were
58
59 275 very similar to the GN ones while the AB (ABnormals Fast Glycolysing) ones exhibited

1
2
3 276 lower intensities for the bands corresponding to MHC and actin proteins. Four AB samples
4
5 277 were easily identified creating a new subgroup representing 20% of the whole GR samples.
6
7
8 278 From these observations, it clearly appears that not all GR samples can be considered as
9
10 279 equivalent, at least for their proteome extracted with high or very high ionic strength buffers.

11
12 280 Results presented in Figure 2 failed to show differences in the quantity of MHC and
13
14 281 actin extracted at 20 min *post-mortem*. However, at 24 h *post-mortem*, WB performed with
15
16 282 antibody against MHC and actin clearly distinguished AB samples from others ones. A larger
17
18 283 quantity of MHC was detected in the proteins extracted from GN samples. AB and GR-AB
19
20 284 samples could also be significantly distinguished. Nevertheless, results from the quantity of
21
22 285 actin extracted at that later time cannot be used to distinguish GN from GR samples or GR-
23
24 286 AB from GN ones, but AB samples exhibited significantly lower amount of actin extracted
25
26
27 287 than in all other samples.

Figures 1 & 2 and Table 1 near here

28
29
30
31 288 Twenty min *post-mortem*, there were few differences between the groups of birds in
32
33 289 the quantity of muscular proteins extracted by the different buffers, thus making the
34
35 290 consequences of an acceleration of the glycolysis *post-mortem* difficult to interpret (Table 1).
36
37 291 Nevertheless, it was noticed that higher quantities of proteins were present in HIS and pellet
38
39 292 extracts coming from GN samples. This indicated that the lowest soluble muscular proteins
40
41 293 were affected by an acceleration in the rate of glycolysis which made them less extractible.
42
43 294 This tended to be more evident if GR samples are distinguished as AB and GR-AB samples
44
45 295 because AB samples presented lower (HIS extracts) or similar values to those found in GR-
46
47 296 AB samples.

48
49
50
51
52 297 After the onset of *rigor mortis* (24 h *post-mortem*), LIS and HIS protein extracts
53
54 298 exhibited higher amounts in GN than in GR samples. This difference between fast and normal
55
56 299 glycolysing samples was also more evident when AB samples were distinguished from GR-
57
58
59
60

1
2
3 300 AB. In that case, all the AB samples were significantly different from the GN ones. This
4
5 301 indicated that AB samples were more distinct to GN than GR-AB ones.
6
7

8 302 Meat quality parameters reported in Table 2 show that initial pH values were similar
9
10 303 in AB and GR-AB samples but lower in GR than in GN ones. In contrast, pH_u values were
11
12 304 similar in all samples and within a normal range. Considering muscle temperature at 20 min
13
14 305 *post-mortem*, results indicated that values for GN, GR and GR-AB samples were similar
15
16 306 (around 42 °C), while values from AB samples were slightly but significantly higher (around
17
18 307 43°C). Considering different water loss values, GR samples presented similar (thawing or
19
20 308 cook loss) or higher (drip losses) values than GN samples. Contrastingly, AB samples had
21
22 309 higher drip and cook loss than GR-AB or GN samples, while thawing ones were similar in all
23
24 310 groups. The processing yields obtained from meat batter preparation were not significantly
25
26 311 different for GN and GR samples, but it was significantly lower for AB than for GR-AB or
27
28 312 GN samples (82.11, 88.95 and 90.40 respectively). After cooking, GN meat samples
29
30 313 presented lower shear force values (F_{max}) than GR ones, but AB and GR-AB samples were
31
32 314 not found to be significantly different. Nevertheless, AB and GR-AB samples were still
33
34 315 significantly tougher than GN ones. Higher L^* values (53.03 ± 2.87) recorded for raw meat
35
36 316 samples also permitted to easily distinguish AB samples from other ones. However, L^* values
37
38 317 were similar in GN and GR samples (49.06 ± 2.92 and 50.14 ± 2.51 respectively). When a^*
39
40 318 values were significantly higher in GR samples than in GN ones, but not in GR-AB ones, they
41
42 319 were also significantly increased in samples coming from the AB group. Table 2 near here

43
44 320 Glycolytic potential (GP) values were similar in all samples (Table 3) suggesting that
45
46 321 muscular reserves in carbohydrates at the time of death were not different between all the
47
48 322 animals. Nevertheless, soon after death, we can observe that glycogen content is higher in GN
49
50 323 than in all other samples. On the contrary, glucose content was lower in GN samples than in
51
52 324 GR ones and was similar in AB and GR-AB samples. Lactate was higher in GR than in GN
53
54
55
56
57
58
59
60

1
2
3 325 samples but failed to distinguish AB to GR-AB samples. No significant differences among all
4
5 326 the animals were recorded for G6P. At the end of the *rigor mortis* onset (24 h *post-mortem*),
6
7 327 similar quantities of all the considered metabolites were measured in all samples.
8
9

10 328 If small significant differences in COX enzyme specific activity (Figure 3) were
11
12 329 recorded in the different samples, a much higher value was reported for the PFK enzyme
13
14 330 specific activity: in GN samples, this value was approximately 6 fold higher than in GR, GR-
15
16 331 AB or AB samples.

Table 3 and Figure 3 near here

332 DISCUSSION

333 Soon after death (20 min *post-mortem*), SDS-Page profiles were similar between all the
334 groups of birds. This demonstrates that the analysed samples were very close in term of
335 proteome composition, as already shown by Pietrzak *et al.* (1997) and Rathgeber *et al.*
336 (1999b), who reported that normal or fast glycosing muscles were not differently organised in
337 terms of constitutive or functional proteins. On the contrary, at the end of the *rigor mortis*
338 onset, SDS-Page profiles were similar for all extracted fractions in GN animals while they
339 allowed us to make a clear difference between the GR ones. This indicates that, despite the
340 fact that all the GR birds were initially identified as being homogenous for the characteristic
341 of the rate of glycolysis (same low initial pH_{20min} values), a clear distinction could be found
342 among them, considering the consequences of this parameter on the solubility of muscular
343 proteins. This distinguishes AB from GR-AB birds. The relevance of the AB group was
344 initially based on differences of SDS-Page profiles for HIS and pellet extracts, mainly
345 because of different intensities for two bands located at 45 and 200 kDa. The identification of
346 the modified bands was made by mass spectrometry analysis and revealed the presence of
347 actin and myosin heavy chain (MHC) at 45 and 200 kDa respectively. Alterations of
348 myofibrillar proteins due to the acceleration of *post-mortem* glycolysis had previously been
349 reported by Rathgeber *et al.* (1999b). Modifications of muscular proteins extractabilities were

1
2
3 350 partly visible at 20 min *post-mortem* because AB samples always differed from GN, but not
4
5 351 completely from GR-AB ones (same value for pellet extract). At 24 h *post-mortem*, AB
6
7 352 samples differed widely not only from GN ones but also from GR-AB, mainly for HIS
8
9
10 353 extracts which contained most of the extractable myofibrillar proteins. By using values of
11
12 354 extractabilities, AB samples could always be distinguishable from GN ones whatever the time
13
14
15 355 *post-mortem* or the considered fraction. In contrast and for all the considered fractions, GR-
16
17 356 AB samples were not always found to be different from the GN ones. Quantitative analysis of
18
19 357 the presence of actin and myosin within the different HIS fractions by WB confirmed that the
20
21 358 level of considered proteins was similar for all the samples at 20 min *post-mortem*. At 24 h
22
23 359 *post-mortem*, there were always significantly smaller amounts of actin and MHC present in
24
25 360 AB samples than in other ones, indicating a different degree of those proteins alteration
26
27 361 between the different samples. Whereas these alterations of actin and MHC in fast
28
29 362 glycolysing muscles have previously been reported by Rathgeber *et al.* (1999b), Alvarado and
30
31 363 Sams (2004) reported no effect of *post-mortem* glycolysis acceleration on the extraction of
32
33 364 myofibrillar proteins, as observed here, if AB samples are excluded from the analysis by
34
35 365 comparing GR-AB with GN ones. This indicates that, depending on the presence or otherwise
36
37 366 of AB samples among GR ones, the occurrence of myofibrillar protein alterations is in
38
39 367 response to accelerated glycolysis *post-mortem*. The presence of an associated increase in
40
41 368 muscle temperature only in AB samples, together with a lack of any difference in pHu values,
42
43 369 suggests that AB samples must be considered as true PSE ones while GR-AB are probably
44
45 370 less affected. The percentage of AB samples among all the GR ones could then explain why
46
47 371 some authors found large effects on meat quality parameters due to accelerated *post-mortem*
48
49 372 glycolysis, while others concluded that the combination low early *post-mortem* pH and high
50
51 373 temperature values had a much smaller effect. It is generally accepted that the combination of
52
53 374 low pH and high temperature values, within muscle, leads to protein alterations (Joo *et al.*,

1
2
3 375 1999; Wilson; van Laack, 1999) and consequently modifies protein extractabilities as well as
4
5
6 376 meat quality (Alvarado and Sams, 2004; Gratacos-Cubarsi and Lametsch, 2008). To our
7
8 377 knowledge, but with the exception of a report by Offer (1991) who estimated the part of
9
10 378 myosin degraded in several pH and temperature conditions, neither the conditions nor the
11
12 379 duration of pH and temperature limit values have precisely been described. As a consequence,
13
14
15 380 we must ask what are the incorrect conditions for meat quality. For example, is a condition
16
17 381 described by a pH value lower than 6.0 and a temperature lower than 40°C during 10 min
18
19 382 worst than one described by a pH value of 6.2 and a temperature higher than 40°C during a
20
21 383 shorter or a longer time? As muscle to meat transformation is a dynamic phenomenon
22
23 384 depending on time, temperature and pH values, we could take into account these three
24
25 385 parameters to better predict consequences on the future of proteins and on associated meat
26
27 386 qualities.

28
29
30
31 387 All data collected to determine the energetic status of sampled muscles confirmed that
32
33 388 AB as well as GR-AB had rapidly started *post-mortem* glycolysis. Indeed, GP level was the
34
35 389 same in all samples, which is in good accordance with observed similar pH_u values because
36
37 390 GP directly reflects the level of stock energy at the time of death (Monin and Sellier, 1985, Le
38
39 391 Bihan-Duval *et al.*, 2008). At the end of the onset of *rigor mortis*, all the recorded values for
40
41 392 energetic compounds were at the same level, indicating that the extent of *post-mortem*
42
43 393 glycolysis was at the same level in all samples, (Warris *et al.*, 1988; El Rammouz *et al.*,
44
45 394 2004). This was in good agreement with final pH_u values. However, whereas glycogen and
46
47 395 glucose levels were higher in GR (including AB and GR-AB samples) than in GN at 20 min
48
49 396 *post-mortem*, the lactate content was the reverse. This demonstrated that glycolysis began
50
51 397 more quickly in GR than in GN muscles, as could be deduced from pH_{20min} values (Shen *et*
52
53 398 *al.*, 2006). In our study, lactate levels, which are mainly responsible for pH fall in *post-*
54
55 399 *mortem* muscles, were similar in AB and GR-AB samples as were pH_{20min} values. PFK

1
2
3 400 enzyme is considered to be a key enzyme in anaerobic glycolysis because it can act as a
4
5 401 regulator of its efficiency. When the level of production of energy (*i.e.* ATP) is high within
6
7 402 the cell, the specific activity of PFK enzyme is reduced to economise the stock of glycogen
8
9
10 403 (Wegener and Krause, 2002). This is probably the case here at 20 min *post-mortem* because,
11
12 404 in GR samples, the mean pH value reported was low, indicating a high level of anaerobic
13
14 405 glycolysis. In contrast, the level of anaerobic glycolysis in GN samples stayed low (as
15
16 406 demonstrated by low levels of lactate and high pH_{20min} values) while the PFK specific activity
17
18 407 remained high. In all samples, low values for specific activities of COX enzyme indicated that
19
20 408 all the samples were glycolytic muscles as previously observed in turkey PM (Opalka *et al.*,
21
22 409 2004). No differences in the specific activity of the PFK enzyme were recorded between AB
23
24 410 and GR-AB samples, demonstrating that a difference in glycolysis regulation should exist
25
26 411 between all the GR and GN samples independently from PFK specific activity modulations. It
27
28 412 is likely that, at the beginning of the slaughtering process, some birds would have enhanced
29
30 413 their muscular glycolysis rate, as did GR, but only few (AB ones) were able to maintain it for
31
32 414 a longer time than the majority of the fast glycolysing ones (here the GR-AB samples). This
33
34 415 would probably lead to the worst combination of pH and temperature values in the muscle
35
36 416 and could explain the poorest final quality of meat (here for AB samples). However, the
37
38 417 origins of this deep disorder in *post-mortem* glycolysis in the muscle is still unknown.

39
40
41
42
43
44
45 418 When we consider meat quality parameters such as drip loss, cooking l, colour values
46
47 419 (mainly L* and a*) and processing yield, our conclusions on the effect of the acceleration of
48
49 420 glycolysis *post-mortem* are completely different if we compare AB, GR-AB or GR samples
50
51 421 with GN. With AB samples, most of the recorded parameters differ from GN, while the final
52
53 422 conclusions are much less evident with GR or GR-AB. For example, higher L* values of meat
54
55 423 in fast-glycolysing samples have been widely discussed, but some workers found this
56
57 424 parameter could be used to sort PSE samples (Barbut, 1997) while others failed to distinguish

1
2
3 425 between them (Molette *et al.*, 2002; Fraqueza *et al.*, 2006). In this study, we demonstrated
4
5 426 that, depending on the origin of samples, AB or GR-AB group, one can find equal or higher
6
7 427 L* values for meat samples with a low initial pH value than in normal ones. If we take as a
8
9 428 reference the cut-off value of L* > 52, as proposed by Barbut (1997), we would sort all the
10
11 429 AB samples as being PSE ones, while all the GR-AB would be considered as the GN ones,
12
13 430 despite that they present very different early pH values. As a consequence, it seems clear that
14
15 431 only measuring one early pH value is not sufficient to accurately detect samples liable to
16
17 432 develop the meat quality alterations generally associated with PSE syndrome in poultry.
18
19
20
21

22 433 **Conclusion**

23
24 434 This paper demonstrates that a simple measurement of one early muscle pH value is probably
25
26 435 insufficient to satisfactorily distinguish turkeys prone to develop PSE syndrome and all the
27
28 436 associated consequences for the quality of meat. Indeed, if the presence of a high muscle
29
30 437 temperature and a low pH value in muscle are required to develop PSE syndrome, the
31
32 438 duration of the conditions encountered *post-mortem* are also fundamental. Further
33
34 439 experiments describing the precise kinetics of pH and temperature evolution *post mortem* are
35
36 440 necessary to adequately understand the etiology of the syndrome. The different possible
37
38 441 combinations of (pH, temperature and duration) could have large consequences on muscle
39
40 442 proteins and variably affect meat quality. Other investigations are also required to understand
41
42 443 why, which and how some turkeys are able to develop such conditions of muscle to meat
43
44 444 transformation leading to the poorest final quality of poultry products.
45
46
47
48
49
50
51

52 446 REFERENCES

- 53
54 447 ALVARADO, C.Z. & SAMS, A.R. (2002) The role of carcass chilling rate in the
55
56 448 development of pale, exudative turkey pectoralis. *Poultry Science*, **81**: 1365-1370.
57
58 449 ALVARADO, C.Z. & SAMS, A.R. (2004) Turkey carcass chilling and protein denaturation
59
60 450 in the development of Pale, Soft and Exudative meat. *Poultry Science*, **83**: 1039-1046.

- 1
2
3 451 BARBUT, S. (1997) Problem of pale soft exudative meat in broiler chickens. *British Poultry*
4 *Science*, **38**: 355-358.
- 5 452
- 6 453 BARBUT, S., SOSNICKI, A.A., LONERGAN, S.M., KNAPP, T., CIOBANU, D.C.,
7
8 454 GATCLIFFE., L.J., HUFF-LONERGAN, E. & WILSON, E.W. (2008) Progress in reducing
9
10 455 the pale, soft and Exudative (PSE) problem in pork and poultry meat. *Meat Science*, **79**: 46-
11
12 456 63.
- 13
14 457 BERGMAYER, H.U., BERNT, E., SCHMIDT, F. & STORK, H. (1974) D-Glucose.
15
16 458 Determination with hexokinase and glucose-6-phosphate dehydrogenase, in: BERGMAYER,
17
18 459 H.U. (Ed.) *Methods of enzymatic analysis*, Vol. 3, pp. 1196-1201 (New York, Academic
19
20 460 Press).
- 21 461 BEUTLER, E. (1971) Phosphofructokinase, in: BEUTLER, E. (Ed) *Red cell metabolism. A*
22
23 462 *manual of biochemical methods*, pp. 42- 44 (New York, Grune and Straton Inc).
- 24 463 BOLES, J.A., PARRISH JR., F.C., HUIATT, T.W. & ROBSON, R.M. (1992) Effect of
25
26 464 porcine stress syndrome on the solubility and degradation of myofibrillar/cytoskeletal
27
28 465 proteins. *Journal of Animal Science*, **70**: 454-464.
- 29
30 466 CHOI, Y.M., RYU, Y.C. & KIM, B.C. (2007) Influence of myosin heavy- and light chain
31
32 467 isoforms on early *post-mortem* glycolytic rate and pork quality. *Meat Science*, **76**: 281-288.
- 33 468 DALRYMPLE, R.H. & HAMM, R. (1973) A method for the extraction of glycogen and
34
35 469 metabolites from a single muscle sample. *Journal of Food Technology*, **8**: 439-444.
- 36
37 470 EL RAMMOUZ, R., BABILÉ R. & FERANDEZ, X. (2004) Effect of ultimate pH on the
38
39 471 physicochemical and biochemical characteristics on turkey breast muscle showing normal rate
40
41 472 of postmortem pH fall. *Poultry Science*, **83**: 1750-1757.
- 42 473 FERNANDEZ, X., SANTÉ, V., BAÉZA, E., LEBIHAN-DUVAL, E., BERRI, C.,
43
44 474 RÉMIGNON, H., BABILÉ, R., LE POTTIER, G. & ASTRUC, T. (2002) Effects of the rate
45
46 475 of muscle pH post mortem pH fall on the technological quality of turkey meat. *British Poultry*
47
48 476 *Science*, **43**: 245-252.
- 49 477 FRAQUEZA, M.J., CARDOSO, A.S., FERREIRA, M.C. & BARRETO, A.S. (2006)
50
51 478 Incidence of pectoralis major turkey muscles with light and dark colour in a Portuguese
52
53 479 slaughterhouse. *Poultry Science*, **85**: 1992-2000.
- 54 480 GRATACOS-CUBARSI, M. & LAMETSCH, R. (2008) Determination of changes in protein
55
56 481 conformation caused by pH and temperature. *Meat Science*, **80**: 545-549.
- 57
58 482 HAHN, G., MALENICA, M. & BRANSCHIED, W. (2002) The relationship between pH
59
60 483 decline *post-mortem* and biochemical and physico-chemical properties of turkey breast meat.
484 *Archiv für Geflügelkunde*, **65**: 66-65.

- 1
2
3 485 HONIKEL, K.O. (1998) Reference methods for the assessment of physical characteristics of
4 meat. *Meat Science*, **49**: 447-457.
- 5 486
6
7 487 JAMES, C., VIANCENT, C., DE ANDRATE LIMA, T.I. & JAMES, S.J. (2006) The primary
8 chilling of poultry carcass – a review. *International Journal of Refrigeration*, **29**: 847-862.
- 9 488
10 489 JEACOCKE, R.E. (1977) The temperature dependence of anaerobic glycolysis in beef muscle
11 held in a linear temperature gradient. *Journal of the Science of Food and Agriculture*, **28**:
12 490
13 491 551-556.
- 14
15 492 JOO, S.T., KAUFFMAN, R.G., KIM, B.C. & PARK, G.B. (1999) The relationship of
16 sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in
17 porcine longissimus muscle. *Meat Science*, **5**: 291-297.
- 18 493
19 494
20 495 KUO, C.C. & CHU, C.Y. (2003) Quality characteristics of Chinese sausages made from PSE
21 pork. *Meat Science*, **64**: 441-449.
- 22 496
23 497 LAEMMLI, U.K. (1970) Cleavage of structural proteins during the assembly of the head of
24 bacteriophage T4. *Nature*, **227**: 680-685.
- 25 498
26 499 LE BIHAN-DUVAL, E., DEBUT, M., BERRI, C.M., SELIER, N., SANTE-
27 L'HOUTELLIER, V., JEGO, Y. & BEAUMONT, C. (2008) Chicken meat quality: genetic
28 variability and relationship with growth and muscle characteristics. *BMC Genetics*, **18**: 9-53.
- 29 500
30 501
31 502 MCKEE, S.R. & SAMS, A.R. (1998) Rigor mortis development at elevated temperature
32 induces pale exudative turkey meat characteristics. *Poultry Science*, **77**: 169-174.
- 33 503
34 504 MOLETTE, C., RÉMIGNON, H. & BABILE, R. (2003) Effect of rate of pH fall on turkey
35 breast meat quality. *British Poultry Science*, **44**: 787-788.
- 36 505
37 506 MOLETTE, C., RÉMIGNON, H. & BABILÉ, R. (2005) Modification of glycolyzing
38 enzymes lowers meat quality of turkey. *Poultry Science*, **84**: 119-27.
- 39 507
40 508 MONIN, G. & SELIER, P. (1985) Pork of low technological quality with a normal rate of
41 muscle pH fall in the immediate post mortem period: The case of the Hampshire breed. *Meat*
42 *Science*, **13**: 49-63.
- 43 509
44 510
45 511 OFFER, G. (1991) Modelling of the formation of Pale, Soft and Exudative meat: Effect of
46 chilling regime and rate and extend of glycolysis. *Meat Science*, **30**: 157-184.
- 47 512
48 513 OPALKA, J.R., WICKE, M., GELLERICH, F.N., SCHMIDT, R., ROSNER, F., ZIERZ, S. &
49 VON LINGERKEN, G. (2004) Mitochondrial function in turkey skeletal muscle--impact on
50 meat quality. *British Poultry Science*, **45**: 367-379.
- 51 514
52 515
53 516 PETRACCI, M., FLETCHER, D.L. & NORTHCUTT, J.K. (2001) The effect of holding
54 temperature on live shrink, processing yield, and breast meat quality of broiler chickens.
55 *Poultry Science*, **80**: 670-675.
- 56 517
57
58
59
60

- 1
2
3 519 PIETRZAK, M., GREASER, M.L. & SOSNICKI, A.A. (1997) Effect of rapid rigor mortis
4 520 processes on protein functionality in *pectoralis major* muscle of domestic turkeys. *Journal of*
5 521 *Animal Science*, **75**: 2106-2116.
- 6
7
8 522 RATHGEBER, B.M., PATO, M.D., BOLES, J.A. & SHAND, P.J. (1999a) Rapid post
9 523 mortem glycolysis and delay chilling of turkey carcasses cause alterations to protein
10 524 extractability and degradation of breast muscle proteins. *Journal of Agricultural and Food*
11 525 *Chemistry*, **47**: 2529-2536.
- 12
13
14 526 RATHGEBER, B.M., BOLES, J.A. & SHAND, P.J. (1999b) Rapid post mortem pH decline
15 527 and delayed chilling reduce quality of turkey breast meat. *Poultry Science*, **78**: 477-484.
- 16
17
18 528 RÉMIGNON, H., MOLETTE, C., EADMUSIK, S. & FERNANDEZ X. (2007) Coping with
19 529 the PSE syndrome in poultry meat. *Proceedings of the XVIII European Symposium on the*
20 530 *Quality of Poultry Meat and the XII European Symposium on the Quality of Eggs and Egg*
21 531 *Products*, Prague.
- 22
23
24 532 SHAND, P.J., SOFOS, J.N. & SCHMIDT, G.R. (1994) Kappa-carrageenan, sodium chloride
25 533 and temperature affect yield and texture of structured beef rolls. *Journal of Food Science*, **49**:
26 534 447-457.
- 27
28
29 535 SHEN, Q.W., MEANS, W.J., UNDERWOOD, K.R., THOMPSON, S.A., ZHU, M.J.,
30 536 McCORMICK, R.J., FORD, S.P., ELLIS, M. & DU, M. (2006) Early *post-mortem* AMP-
31 537 activated protein kinase (AMPK) activation leads to phosphofructokinase-2 and -1 (PFK-2
32 538 and PFK-1) phosphorylation and the development of pale, soft, and exudative (PSE)
33 539 conditions in porcine longissimus muscle. *Journal of Agriculture and Food Chemistry*, **54**:
34 540 5583-5589.
- 35
36
37 541 SMITH, L. & CONRAD, H. (1956) A study of the kinetics of the oxidation of cytochrome c
38 542 by cytochrome c oxidase. *Archives of Biochemistry and Biophysics*, **63**: 403-413.
- 39
40
41 543 TOWBIN, H., STAHELIN, T. & GORDON, J. (1979) Electrophoresis transfer of proteins
42 544 from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications.
43 545 *Proceedings of the National Academy of Science (USA)*, **76**: 4350-4354.
- 44
45
46 546 WARNER, R.D., KAUFFMAN, R.G. & GREASER, M.L. (1997) Muscle protein changes
47 547 *post-mortem* in relation to pork quality traits. *Meat Science*, **45**: 339-352.
- 48
49
50 548 WARRIS, P.D. & BROWN, S.N. (1987) The relationships between initial pH, reflectance and
51 549 exudation in pig muscle. *Meat Science*, **20**: 65-74.
- 52
53
54 550 WARRIS, P.D., KESTIN, S.C., BROWN, S.N. & BECIS, E.A. (1988) Depletion of glycogen
55 551 reserves in fasting broiler chickens. *British Poultry Science*, **29**: 149-154.

- 1
2
3 552 WEGENER G. & KRAUSE U. (2002) Different modes of activating phosphofructokinase, a
4
5 553 key regulatory enzyme of glycolysis, in working vertebrate muscle. *Biochemical Society*
6
7 554 *Transactions*, **30**: 264-270.
- 8
9 555 WILSON, G.G.I. & VAN LAACK, R.L.J.M. (1999) Sarcoplasmic proteins influence water-
10
11 556 holding capacity of pork myofibrils. *Journal of the Science of Food and Agriculture*, **79**:
12
13 557 1939-1942.
- 14 558 WYNVEEN, E.J., BOWKER, B.C., GRANT, A.L., DEMOS, B.P. & GERRARD, D.E.
15
16 559 (1999) Effects of muscle pH and chilling on development of PSE-like turkey breast meat.
17
18 560 *British Poultry Science*, **40**: 253-256.
- 19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

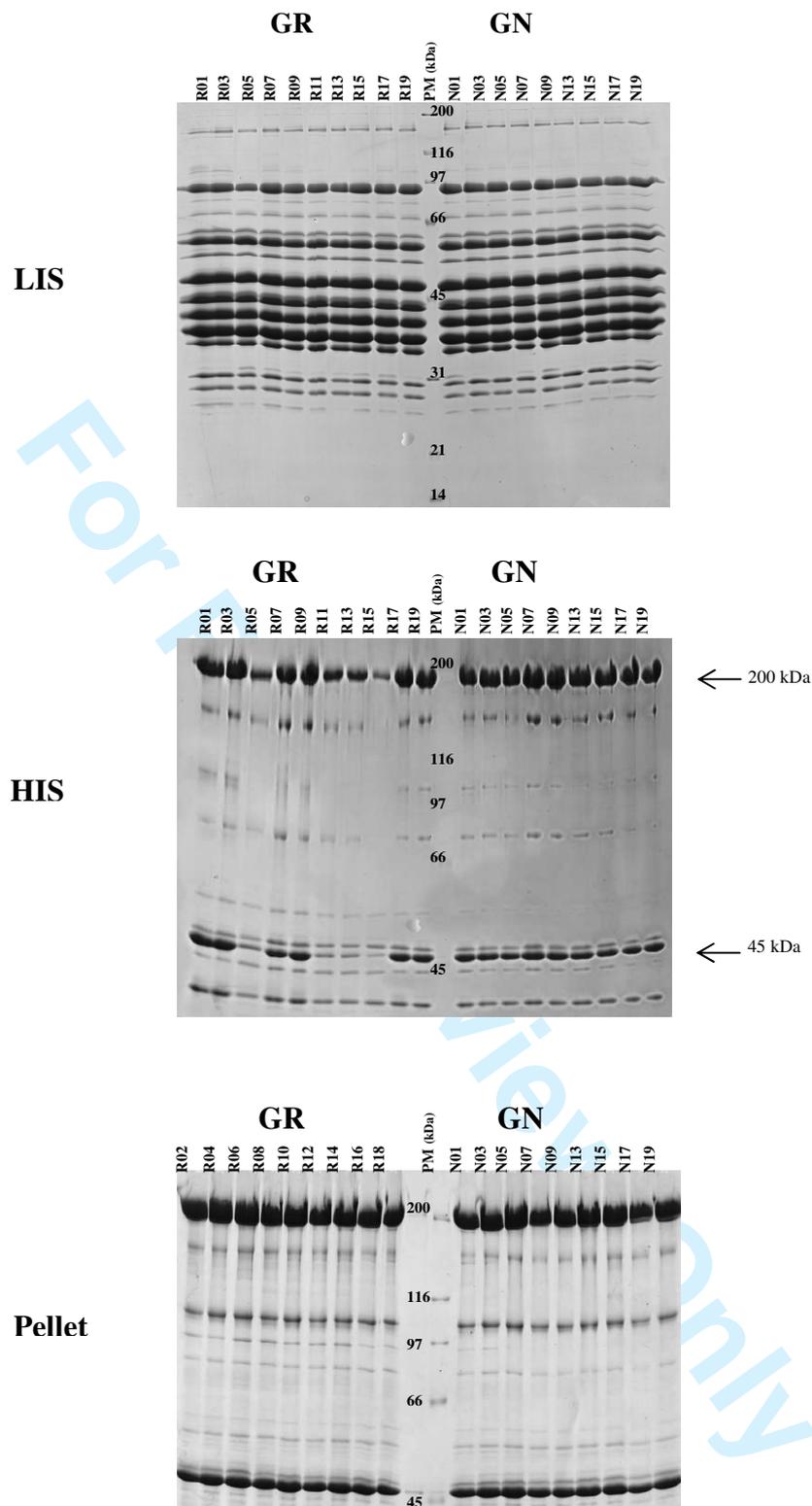


Figure 1. Examples of SDS-Page gels from muscular proteins extracted with different buffers (LIS=Low Ionic Strength, HIS=High Ionic Strength, Pellet= residue after Lis and His buffers extractions) at 24 h post mortem from GR (Fast Glycolysing) and GN (Normal Glycolysing) turkeys. One different animal is present per lane and arrows indicate major variation in band staining.

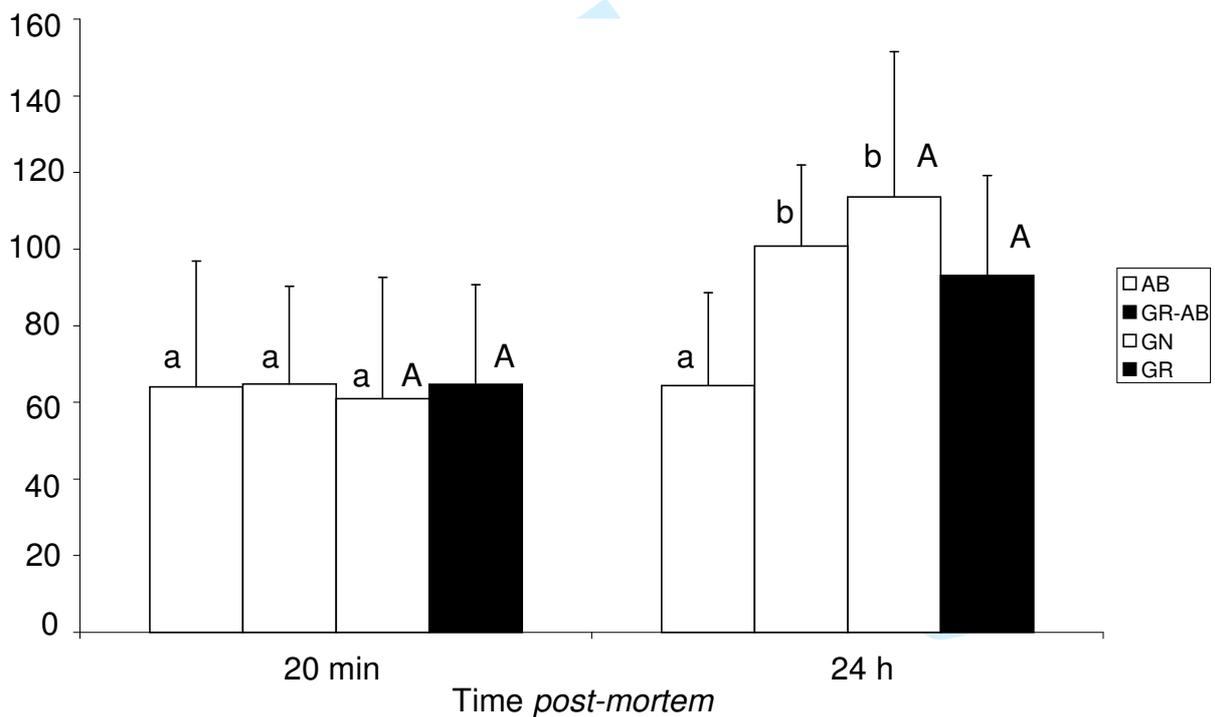
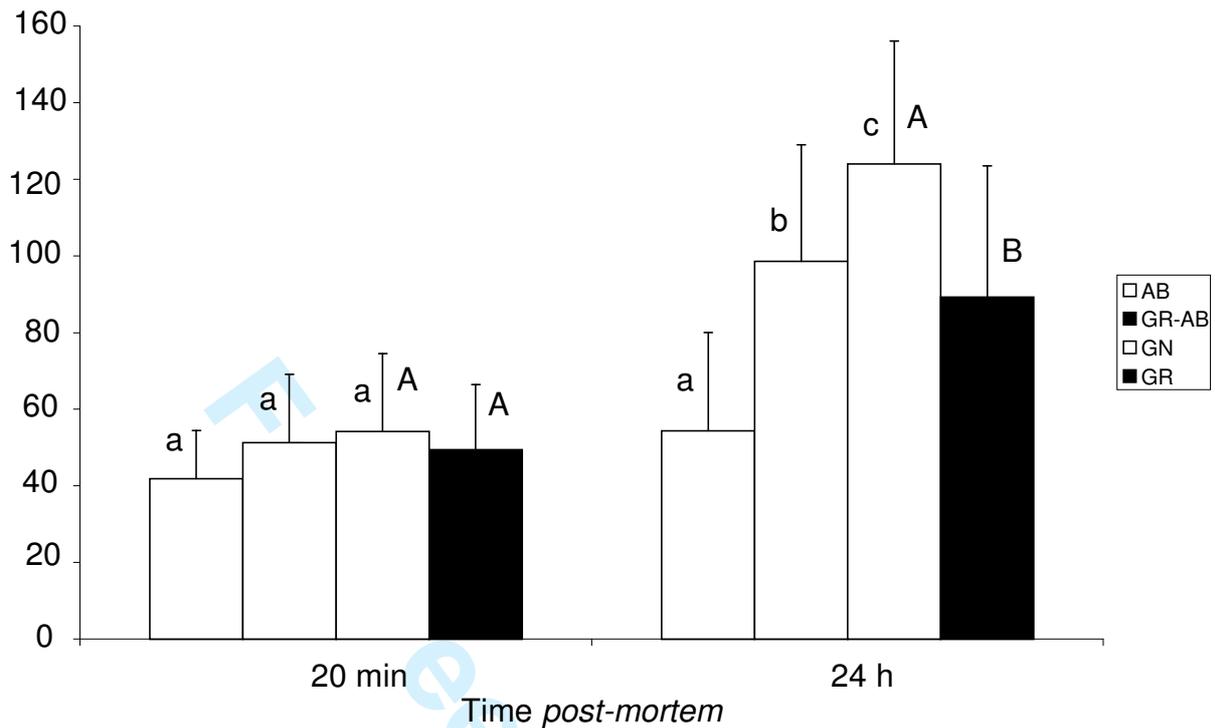
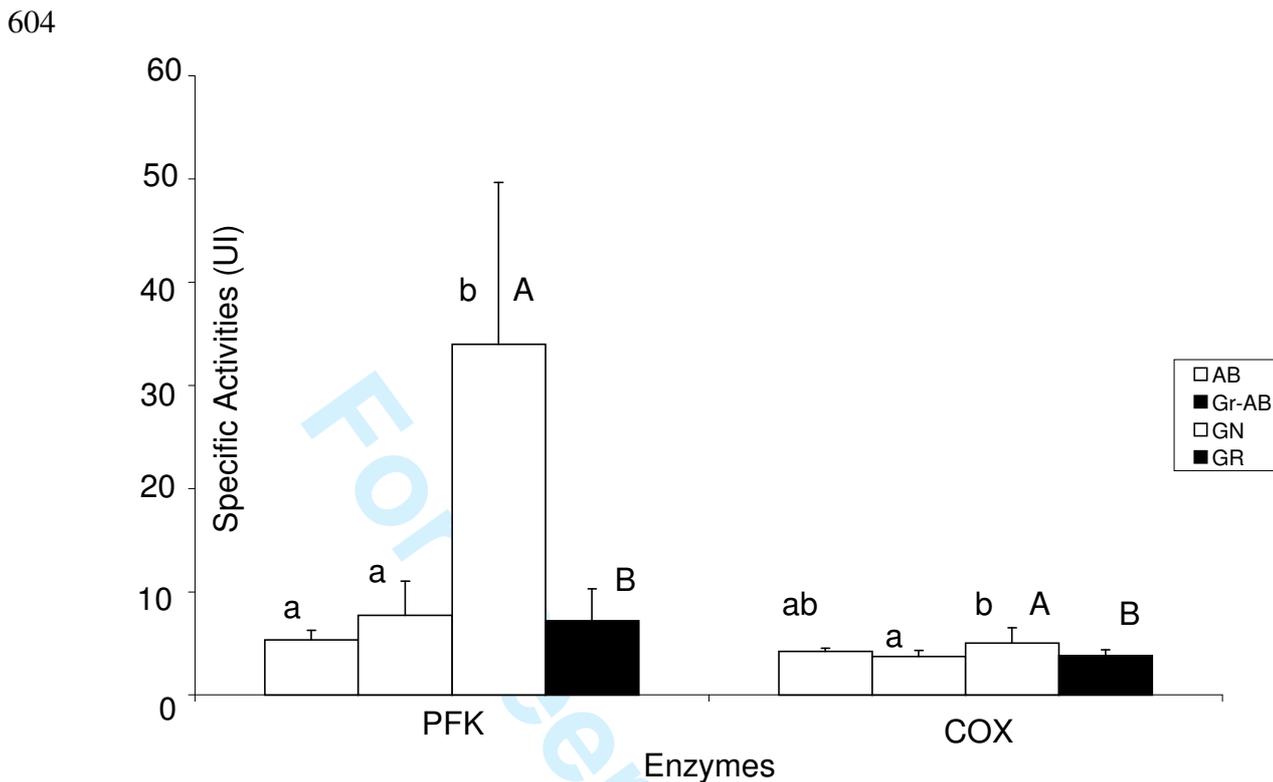


Figure 2. Western blot values with antibody against Myosin Heavy Chain (top) and actin (bottom) at 20 min and 24 h post mortem for the different groups of turkeys. Values are means \pm SD and are expressed as percentages of reference protein. GN = Normal Glycolysing, GR = Fast Glycolysing, AB = Abnormal GR, GR-AB = GR animals not AB. ^{a,b}AB, GR-AB and GN means with different superscripts differ significantly at $P < 0.05$, ^{A,B}GN and GR means with different superscripts differ significantly at $P < 0.05$.



29 605
30 606 **Figure 3.** Specific activities (in UI) of the PFK (PhosphoFructoKinase) and cytochrome
31 607 oxydase c (COX) enzymes in the different groups of turkeys. Values are means \pm SD. GN =
32 608 Normal Glycolysing, GR = Fast Glycolysing, AB = Abnormal GR, GR-AB = GR birds not
33 609 AB. ^{a,b}AB, GR-AB and GN means with different superscripts differ significantly at $P < 0.05$,
34 610 ^{A,B}GN and GR means with a different superscript differ significantly at $P < 0.05$.

611 **Table 1.** Percentage of proteins extracted by buffer with increasing ionic strength at 20 min
 612 or 24 h post-mortem in different groups of turkeys. Values are means \pm SD and expressed as
 613 percentages of total proteins

Time <i>post mortem</i>	%	AB	GR-AB	GN	GR
20 min	LIS	22.7 \pm 2.38 ^a	25.0 \pm 0.92 ^{ab}	25.0 \pm 1.03 ^b	24.8 \pm 0.93
	HIS	5.9 \pm 1.03 ^a	7.0 \pm 0.78 ^b	7.6 \pm 1.17 ^{b,A}	6.8 \pm 1.00 ^B
	Pellet	23.2 \pm 5.10 ^a	26.3 \pm 3.33 ^a	30.7 \pm 2.87 ^{b,A}	26.3 \pm 3.09 ^B
24 h	LIS	23.6 \pm 0.69 ^a	27.0 \pm 1.67 ^b	29.3 \pm 1.13 ^{c,A}	26.3 \pm 2.05 ^B
	HIS	5.4 \pm 0.95 ^a	16.6 \pm 4.45 ^b	18.2 \pm 4.67 ^{b,A}	14.4 \pm 6.00 ^B
	Pellet	32.5 \pm 5.46 ^a	27.8 \pm 6.01 ^{ab}	27.0 \pm 4.36 ^b	28.8 \pm 6.07

614 ^{a,b}AB, GR-AB and GN means with different superscripts differ significantly at $P < 0.05$.

615 ^{A,B}GN and GR means with different superscripts differ significantly at $P < 0.05$.

616 GN = Normal Glycolysing, GR = Fast Glycolysing, AB = Abnormal GR, GR-AB = GR
 617 turkeys not AB. LIS = Low Ionic Strength buffer, His = High Ionic Strength buffer, Pellet =
 618 residue after Lis and His buffers extractions.

619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660

621 **Table 2.** *Meat quality parameters in different groups of turkeys. Values are means \pm SD*

	AB	GR-AB	GN	GR
pH ₂₀	5.8 \pm 0.12 ^a	5.8 \pm 0.39 ^a	6.2 \pm 0.01 ^{b,A}	5.8 \pm 0.07 ^B
pH _u	5.8 \pm 0.03	5.8 \pm 0.09	5.8 \pm 0.09	5.8 \pm 0.08
T ₂₀ (°C)	43.1 \pm 2.22 ^a	41.7 \pm 0.91 ^{ab}	41.7 \pm 0.66 ^b	42.1 \pm 1.37
Drip losses (%)	6.2 \pm 1.47 ^a	3.7 \pm 1.06 ^b	3.2 \pm 0.08 ^{b,A}	4.2 \pm 1.45 ^B
Thawing losses (%)	4.8 \pm 0.33	4.5 \pm 2.35	4.0 \pm 1.79	4.5 \pm 2.07
Cooking losses (%)	17.7 \pm 1.24 ^a	14.4 \pm 2.05 ^b	14.2 \pm 2.17 ^b	15.1 \pm 2.34
F _{max} (N)	43.5 \pm 14.83 ^a	30.8 \pm 11.04 ^a	25.0 \pm 4.25 ^{b,A}	33.4 \pm 12.57 ^B
L*	53.0 \pm 2.87 ^a	49.4 \pm 1.89 ^b	49.1 \pm 2.92 ^b	50.1 \pm 2.51
a*	7.3 \pm 1.04 ^a	5.5 \pm 1.147 ^b	5.1 \pm 0.60 ^{b,A}	5.9 \pm 1.34 ^B
b*	0.76 \pm 1.31	0.14 \pm 0.85	0.19 \pm 0.64	0.26 \pm 0.92
Processing yield (%)	82.1 \pm 1.34 ^a	89.0 \pm 4.17 ^b	90.4 \pm .14 ^{b,A}	87.6 \pm 4.68 ^B

622 ^{a,b}AB, GR-AB and GN means with different superscripts significantly differ at $P < 0.05$.

623 ^{A,B}GN and GR means with different superscripts significantly differ at $P < 0.05$.

624 GN = Normal Glycolysing, GR = Fast Glycolysing, AB = Abnormal GR, GR-AB = GR
625 turkeys not AB.
626

Table 3. Main energetic metabolites contain in Pm muscle of turkeys from the different groups at 20 min and 24 h post-mortem. Values are means \pm SD and are expressed in μ moles/g tissue except for the glycolytic potential (GP) values which are expressed in μ moles of lactate/g tissue

Time <i>post mortem</i>		AB	GR-AB	GN	GR
20 min	Glycogen	1.8 \pm 1.42 ^a	6.4 \pm 3.48 ^b	14.1 \pm 5.02 ^{c,A}	5.5 \pm 3.67 ^B
	G6P	0.34 \pm 0.29	0.20 \pm 0.20	0.21 \pm 0.22	0.23 \pm 0.22
	Glucose	2.6 \pm 0.57 ^a	2.5 \pm 0.55 ^a	1.6 \pm 0.37 ^{b,A}	2.5 \pm 0.54 ^B
	Lactate	102.8 \pm 11.19 ^a	98.8 \pm 6.80 ^a	78.1 \pm 6.03 ^{b,A}	99.6 \pm 7.68 ^B
24 h	Glycogen	0.27 \pm 0.15	0.71 \pm 0.52	0.79 \pm 0.66	0.62 \pm 0.50
	G6P	0.82 \pm 0.52	2.03 \pm 2.08	2.29 \pm 1.43	1.77 \pm 1.91
	Glucose	2.7 \pm 0.99	2.4 \pm 0.82	2.2 \pm 0.82	2.5 \pm 0.84
	Lactate	115.2 \pm 9.03	115.7 \pm 6.44	114.9 \pm 10.13	115.6 \pm 6.78
	GP	122.7 \pm 11.66	126.0 \pm 9.88	125.3 \pm 13.54	125.3 \pm 9.96

^{a,b} Means with different superscript significantly differ ($P < 0.05$) between AB, GR-AB and GN.

^{A,B} Means with different superscript significantly differ ($P < 0.05$) between GN and GR.

GN = Normal Glycolysing, GR = Fast Glycolysing, AB = Abnormal GR, GR-AB = GR turkeys not AB.