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

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Are one early muscle pH and one early temperature measurement sufficient to detect PSE breast meat in turkeys?

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Abstract 1. Within a large flock of turkey toms (2000 BUT9 conventionally reared and slaughtered), early muscle pH measurements were randomly done to distinguish two groups of birds presenting low (fast glycolysing, GR) or normal (normal glycolysing, GN) values.

2. Subsequently, ultimate pH values and meat quality parameters were also recorded. Meat quality parameters from GR or GN samples differ more or less indicating more or less severe PSE conditions. Proteins extracted from the samples at 20 min post mortem were quite similar while they greatly differ at 24 h post mortem. Moreover, among the GR birds, a subgroup of animals (called AB) presented SDS-PAGE profiles largely different from other GR or GN birds.

3. All the subsequent analysis developed on meat quality parameters as well as for protein extractabilities also differ between AB and other animals indicating that they must be considered differently in term of PSE syndrome development.

4. Western blots against Myosin Heavy Chain and actin at 24 h post mortem indicate that myofibrillar proteins alterations are different in AB and GR or GN samples.

5. At 20 min post mortem, glycogen content was lowest in AB samples while the glycolytic potential was similar in all samples at the time of death. Measurements of PFK enzyme specific activity did not indicate a different regulation of post mortem glycolysis in AB samples.

6. Our results suggest that a unique pH measurement at 20 min post is insufficient to detect animals more prone to developing a severe PSE syndrome in turkeys. In consequence, it is suggested that a more precise evaluation of the kinetics of pH and temperature decrease has to be conducted to understand the aetiology of meat quality parameters alterations in poultry.

INTRODUCTION

Since the beginning of the 1990's, the PSE (Pale, Soft and Exudative) syndrome has been given a lot of attention from poultry scientists, as well as meat processors, because of its

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

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23
24 60 Several facets of the production of live birds and/or management of carcasses have been
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26 61 investigated, mainly in turkeys and chickens (for review, Barbut *et al.*, 2008), to describe the
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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*
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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
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36 66 low pH values at any time soon after slaughter, when the temperature of muscles is still high
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38 67 (Petracci *et al.*, 2001). Several experiments have then been conducted to maintain poultry
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40 68 muscle at high *post-mortem* temperatures and these have shown that such conditions lead to
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42 69 poor meat quality if the rate of pH fall is not concomitantly reduced (Molette *et al.*, 2003;
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44 70 Alvarado and Sams, 2002; Rathgeber *et al.*, 1999b). Studies have also been conducted to sort
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46 71 animals according to their early muscle pH value and have demonstrated that fast glycolysing
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48 72 muscles (*i.e.* with a low early muscle pH value) are generally associated with poor meat
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50 73 qualities similar to those described when PSE or PSE-like syndrome develops (Warris and
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52 74 Brown, 1987; Pietrzak *et al.*, 1997; Rathgeber *et al.*, 1999a; Wynveen *et al.*, 1999; James *et*
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54 75 *al.*, 2006). Nevertheless, all these studies have observed the consequence of the presence of

both low pH and high temperature in poultry muscles at early times *post-mortem*, but no one has yet proposed a clear origin of those conditions.

The measurement of early pH values cannot be generalised in processing plants because it is destructive and not easy to perform. Soon after death, plasma membranes are still in their native form and most of the water present in the muscle is retained in the myofibrillar structure. Therefore, to have a good estimation of early pH values, it is absolutely necessary to grind muscle samples in a buffer and to be able immediately to stop the anaerobic glycolysis (iodoacetate is generally used as recommended by Jeacocke, 1977). On the contrary, after the setup of *rigor mortis*, a large part of the water have been expelled from the myofibrillar structure and released into the extracellular spaces after plasma membrane disruptions. Thus, a direct measurement of proton concentration is possible with an adapted (meat-type) pH electrode.

In consequence, although numerous studies are available on the variability of ultimate pH (pHu) values within a given flock (Fernandez *et al.*, 2002; Hahn *et al.*, 2002; Fraqueza *et al.*, 2006), few have paid attention to this variability in pH measurements made soon after the birds have been slaughtered. Because muscle pH monitoring is not easily performed on a slaughter line, authors generally report only one early pH value (recorded at 15 or 20 min *post-mortem*) to define characteristics of fast or normal glycolysing muscles. Nevertheless, we must consider that *post-mortem* glycolysis is a dynamic phenomenon which cannot be described by a single measurement. The complexity of these measurements is increased if the monitoring of internal muscle temperature has also to be taken into account.

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

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

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11
12 105 In the present study, we investigated whether all turkeys which exhibit a low early pH
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14 106 value (20 min *post-mortem*) must be considered as a whole or if some of them could exhibit
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16 107 different protein alterations which could make them more or less typical of the PSE
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18 108 syndrome. To be sure that all the finally observed meat quality alterations were only due to
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20 109 different glycolytic rates, all the birds were initially randomly selected from a commercial
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22 110 flock with special attention being given to ultimate pH value (pHu) and glycogen reserves to
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24 111 ensure that the amplitude of the decline in pH did not interact with the rate of pH fall.
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29 112 MATERIALS AND METHODS

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31 113 **Animals**

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33 114 A flock of 2000 turkey toms (line BUT9, BUT Ltd., Chester, UK), was reared in a
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35 115 commercial farm to 19 weeks of age (average live weight = 15 kg). All the birds were fasted
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37 116 for 10 h before slaughter and then conventionally processed in a slaughter-house (Gastronome
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39 117 SA, Moncoutant, France).
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43 118 **Sampling**

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45 119 At the end of the slaughtering process, the pH-values of 458 breast muscles (*Pectoralis major*,
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47 120 Pm) were randomly measured (pH_{20min}) according to Jeacocke (1977). Two grams of breast
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49 121 muscle were homogenised in 18 ml of 5 mM iodoacetate using an Ultra Turrax (IKA-
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51 122 WERKE, Staufen, Germany). The pH_{20min} was then measured by using a portable pH-meter
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53 123 combined with a glass electrode. The internal breast muscle temperature was also measured at
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55 124 20 min *post-mortem* and 20 g of muscle were collected and immediately frozen in liquid
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57 125 nitrogen.
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Two groups were then sorted according to their $\text{pH}_{20\text{min}}$ values from all the measured animals: the first one (Normal Glycolysis, GN, $n = 20$) was made up with animals showing $\text{pH}_{20\text{min}}$ values similar to the mean value (6.21 ± 0.01) of the whole population, while the second one (Rapid Glycolysis, GR, $n = 20$) was made up with birds presenting the lowest $\text{pH}_{20\text{min}}$ values (5.80 ± 0.07). Following storage overnight at 4°C , pH_u was then directly measured by inserting a glass pH-probe into the Pm muscle in GN and GR samples. At 24 h *post-mortem*, other Pm samples were harvested as described above and kept at -80°C until analysed.

Meat quality measurements

Colour

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

Water-holding capacity

Drip loss was evaluated on scallops (around 100 g) of breast muscle collected 1 d *post-mortem*. The scallops were then stored for 9 d at 4°C in a polystyrene tray covered with a scattering film (air permeable). After final weighing, drip loss was expressed as a percentage of the initial weight at 1 d (Honikel, 1998). After 9 d of storage, the scallops were weighed (pre-thaw weight) and frozen at -20°C for 2 weeks. They were allowed to thaw overnight at 4°C and were then reweighed (precook weight). Thaw loss was expressed as a percentage of the pre-thawing weight. The thawed meat was further used to evaluate cook loss and sausage yield. The scallops were vacuum-packaged and cooked in an 80°C water bath for 15 min. After cooking, samples were then cooled down to room temperature, rapidly wiped and weighed. Cooking loss was expressed as a percentage of the precooking weight.

Meat batter yield

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3 151 Sausages were produced following the method described by Shand *et al.* (1994). Thawed
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5 152 meat (52.5g) was trimmed, chopped and mixed with iced water (21g, 0°C) and salt (1.5g) for
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8 153 25 s using a Moulinex ILLICO (Lyon, France) blender. The meat batter was then transferred
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10 154 to a plastic bag and vacuum packaged (residual pressure < 10mb) to remove air bubbles.
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12 155 Twenty grams of meat batter were poured into a polypropylene tube and centrifuged at 700 *g*
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15 156 at 4°C for 10 min. The tubes were then placed in a hot water bath until the internal
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17 157 temperature of meat paste reached 80°C. After cooling down, the tubes were removed and the
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19 158 sausages were weighed. The yield was expressed as a percentage of initial weight.
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22 159 *Warner-Bratzler shear test*
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24
25 160 The texture of cooked meat was evaluated according to Honikel (1998). A 1-cm-wide strip of
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27 161 cooked meat was obtained by cutting parallel to the longitudinal axis of the scallop myofibres.
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29 162 Shear force was measured using a MTS Synergie 200 apparatus (MTS, Eden Prairie, MN,
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31 163 USA). The maximum force needed to shear the sample represented the meat tenderness and
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33 164 was expressed in Newtons.
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36 165 *Protein extractability*
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39 166 Muscular proteins of Pm samples collected at 20 min and 24 h *post-mortem* were extracted
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41 167 following the methods of Rathgeber *et al.* (1999a) and Boles *et al.* (1992). One gram of
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43 168 sample was homogenised in 20 ml of a low ionic strength (LIS) buffer, containing 0.05 M
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45 169 potassium phosphate buffer, 1 mM NaN₃ and 2 mM EDTA/pH 7.3 at 2°C for 10 sec. The
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47 170 homogenised sample was then agitated for 30 min at 4°C and centrifuged at 17500 *g* at 2°C
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49 171 for 15 min. Ten millilitres of the supernatant (LIS fraction) were collected at 2 cm above the
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51 172 bottom of the tube while the remaining supernatant was discarded. A supplemental 20 ml LIS
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53 173 buffer was added to resuspend the pellet which was then homogenised, agitated and
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55 174 centrifuged as previously describe. The supernatant was discarded and the procedure was
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57 175 repeated with high ionic strength (HIS) buffer, containing 0.55 M KCl, 0.05 M potassium
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phosphate buffer, 1 mM NaN₃ and 2 mM EDTA/pH 7.3 at 2°C. Following centrifugation, 10 ml of supernatant (HIS fraction) was collected as described above and the excess supernatant removed. The pellet was re-suspended in 40 ml of potassium phosphate buffer containing 75 mM KCl, 10 mM KH₂PO₄, 2 mM MgCl₂ and 2 mM EGTA/pH 7 and sonicated (Sonificateur, Bioblock Scientific, Illkirch, France) for 1 min to solubilise the fraction (pellet fraction). Protein content in LIS, HIS and pellet fractions were determined by the BCA protein assay kit (PIERCE, Rockford, IL, USA). Results are expressed as a percentage of total proteins.

Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed in order to separate proteins in each buffer-extracted fraction from samples collected at 20 min and 24 h *post-mortem*. Samples containing 2% (w/v) SDS, 5% β-mercaptoethanol, 10% glycerol and 62 mM Tris-HCl, pH 6.8 in a ratio 1:1 v/v (Laemmli, 1970) were heated at 95°C for 5 min. SDS-PAGE was performed following the method described by Laemmli (1970) using a Mini-Protean II electrophoresis unit or a Protean Xi Unit (Bio-Rad Laboratories Inc., Hercules, CA, USA). Samples were loaded at 40 µg protein per lane for LIS, HIS and pellet fractions, 5 and 10 µg for western blots of actin and Myosin Heavy Chain (MHC), respectively. Resolving gel of 12% polyacrylamide was used for LIS fraction and 8% polyacrylamide was applied for HIS and pellet fractions and western blots of actin and MHC. Gels were run at 17 mA or 35 mA/gel (for Mini-Protean II and Protean Xi Units, respectively), constant current, until the dye front reached the bottom of the gel. Gels were stained overnight in Coomassie brilliant blue R-250 containing 5% (v/v) glacial acetic acid, and 45% (v/v) ethanol. Gels were then destained twice in the same solution excluding Coomassie blue.

Western blots of Actin and Myosin Heavy Chain

HIS fraction was used to perform western blots of actin and MHC. Following SDS-PAGE, gel was steeped into transfer buffer containing 25 mM Tris, 192 mM glycine and 20% v/v

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3 201 methanol for 20 min (Towbin *et al.*, 1979). Proteins were transferred to nitrocellulose
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5 202 membrane (Hybond ECL, Amersham, Munich, Germany) at 30 V overnight using a transfer
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8 203 cell (EBU 4000, CBS scientific, CA, USA). Membrane was blocked with milk buffer
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10 204 containing 3% w/v milk powder in phosphate buffer saline (PBS, pH 7.5) at room temperature
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12 205 (RT) for 1 h. Following blocking, membrane was probed for specific proteins using primary
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14 206 antibody (mouse anti-actin, MAB 1501, Chemicon, CA, USA; mouse anti-myosin, F59,
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17 207 DSHB, IA, USA) at RT for 2 h. The primary antibody to milk buffer ratio was 1:1000 and
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19 208 1:25 for actin and MHC respectively. The membrane was then washed 3 times with PBS and
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21 209 incubated in secondary antibody (goat anti-mouse IgG, AP 127, Chemicon, CA, USA) at RT
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23 210 for 1.5 h. The secondary antibody to milk buffer ratio was 1:50,000 for actin and 1:25,000 for
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25 211 MHC. Following this incubation, the membrane was washed 3 times and the
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27 212 chemiluminescent substrate (Super Signal® West Pico, PIERCE) used to detect the reactivity
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29 213 of the primary antibody with its antigen.
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34 214 The Western blot was photographed by making a contact between the membrane and a
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36 215 photo film (Amersham HyperfilmMP, GE Healthcare, Munich, Germany). The film was then
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38 216 developed in 10% revelation solution (AL4, Kodak, NY, USA), distilled water and 10%
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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
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44 219 respectively. Band intensity of samples was then measured by using Image Analysis
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46 220 (ImageMaster 2D Platinum 6.0, GE Healthcare, NJ, USA). It was expressed as a percentage
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48 221 of reference protein (actin and MHC of chicken breast muscle).
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53 222 **Parameters of energetic metabolism**

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55 223 *Assays of phosphofructokinase (PFK) and Cytochrome c Oxidase (COX) activities*

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57 224 About 200 mg of Pm muscle was collected at 20 min *post-mortem* and homogenised with 4
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59 225 ml of buffer containing 0.25 M sucrose, 2 mM EDTA and 10 mM Tris-HCL (pH 8.0) for 30 s
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at 4°C. The enzyme extract was obtained from centrifugation at 10000 g for 5 min at 4°C. The supernatant was collected and stored at 0°C. Activity of PFK was spectrophotometrically measured in the presence of pyruvate kinase (PK) and lactate dehydrogenase (LDH) by using a UVIKON 943 spectrometer (Kontron Instruments, Watford, UK) to follow a disappearance of co-substrate (NADH) at 340 nm and 25°C for 2 min (Beutler, 1971). The reaction was assayed by mixing 1 µl of enzyme extract with 1 ml of solution containing 0.07 M Tris-HCl (pH 8), 1.4 mM MgCl₂, 4.5 mM KCL, 0.71 mM phosphophenol pyruvate, 0.65 mM fructose-1,6- diphosphate, 0.4 mM NADH, 1 mM ATP, 1.8 mM fructose-6-phosphate, 0.08 U PK and 0.96 U LDH. The PFK activity was expressed as µmol of NADH disappeared per min and per g of muscle (µmol/min/g).

The preparation of enzyme extract for COX activity was performed as described above except that, after centrifugation, the supernatant was frozen and defrosted 4 times in liquid nitrogen to break cell wall and to release the enzyme contained in the mitochondrial matrix. The enzyme extract was kept at -20°C until used. Twenty µl of enzyme extract were mixed with 1 ml of solution (pH 7.4) containing 90 µM Cytochrome c (reduced form) and 50 mM potassium phosphate (Smith and Conrad, 1956). The activity was assayed at 550 nm and 25°C. The COX activity was expressed as micromoles of Cytochrome c (reduced form) disappeared per min and per g of muscle .

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

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

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

17 257 **Statistics**

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

36 265 **RESULTS**

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

lower intensities for the bands corresponding to MHC and actin proteins. Four AB samples were easily identified creating a new subgroup representing 20% of the whole GR samples. From these observations, it clearly appears that not all GR samples can be considered as equivalent, at least for their proteome extracted with high or very high ionic strength buffers.

Results presented in Figure 2 failed to show differences in the quantity of MHC and actin extracted at 20 min *post-mortem*. However, at 24 h *post-mortem*, WB performed with antibody against MHC and actin clearly distinguished AB samples from others ones. A larger quantity of MHC was detected in the proteins extracted from GN samples. AB and GR-AB samples could also be significantly distinguished. Nevertheless, results from the quantity of actin extracted at that later time cannot be used to distinguish GN from GR samples or GR-AB from GN ones, but AB samples exhibited significantly lower amount of actin extracted than in all other samples.

Figures 1 & 2 and Table 1 near here

Twenty min *post-mortem*, there were few differences between the groups of birds in the quantity of muscular proteins extracted by the different buffers, thus making the consequences of an acceleration of the glycolysis *post-mortem* difficult to interpret (Table 1). Nevertheless, it was noticed that higher quantities of proteins were present in HIS and pellet extracts coming from GN samples. This indicated that the lowest soluble muscular proteins were affected by an acceleration in the rate of glycolysis which made them less extractible. This tended to be more evident if GR samples are distinguished as AB and GR-AB samples because AB samples presented lower (HIS extracts) or similar values to those found in GR-AB samples.

After the onset of *rigor mortis* (24 h *post-mortem*), LIS and HIS protein extracts exhibited higher amounts in GN than in GR samples. This difference between fast and normal glycolysing samples was also more evident when AB samples were distinguished from GR-

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2
3 300 AB. In that case, all the AB samples were significantly different from the GN ones. This
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5 301 indicated that AB samples were more distinct to GN than GR-AB ones.
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8 302 Meat quality parameters reported in Table 2 show that initial pH values were similar
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10 303 in AB and GR-AB samples but lower in GR than in GN ones. In contrast, pH_u values were
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12 304 similar in all samples and within a normal range. Considering muscle temperature at 20 min
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14 305 *post-mortem*, results indicated that values for GN, GR and GR-AB samples were similar
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16 306 (around 42 °C), while values from AB samples were slightly but significantly higher (around
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18 307 43°C). Considering different water loss values, GR samples presented similar (thawing or
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20 308 cook loss) or higher (drip losses) values than GN samples. Contrastingly, AB samples had
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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
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26 311 different for GN and GR samples, but it was significantly lower for AB than for GR-AB or
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28 312 GN samples (82.11, 88.95 and 90.40 respectively). After cooking, GN meat samples
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30 313 presented lower shear force values (F_{max}) than GR ones, but AB and GR-AB samples were
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32 314 not found to be significantly different. Nevertheless, AB and GR-AB samples were still
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34 315 significantly tougher than GN ones. Higher L^* values (53.03 ± 2.87) recorded for raw meat
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36 316 samples also permitted to easily distinguish AB samples from other ones. However, L^* values
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38 317 were similar in GN and GR samples (49.06 ± 2.92 and 50.14 ± 2.51 respectively). When a^*
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40 318 values were significantly higher in GR samples than in GN ones, but not in GR-AB ones, they
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42 319 were also significantly increased in samples coming from the AB group. Table 2 near here
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51 320 Glycolytic potential (GP) values were similar in all samples (Table 3) suggesting that
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53 321 muscular reserves in carbohydrates at the time of death were not different between all the
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55 322 animals. Nevertheless, soon after death, we can observe that glycogen content is higher in GN
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57 323 than in all other samples. On the contrary, glucose content was lower in GN samples than in
58
59 324 GR ones and was similar in AB and GR-AB samples. Lactate was higher in GR than in GN
60

samples but failed to distinguish AB to GR-AB samples. No significant differences among all the animals were recorded for G6P. At the end of the *rigor mortis* onset (24 h *post-mortem*), similar quantities of all the considered metabolites were measured in all samples.

If small significant differences in COX enzyme specific activity (Figure 3) were recorded in the different samples, a much higher value was reported for the PFK enzyme specific activity: in GN samples, this value was approximately 6 fold higher than in GR, GR-AB or AB samples.

Table 3 and Figure 3 near here

DISCUSSION

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

partly visible at 20 min *post-mortem* because AB samples always differed from GN, but not completely from GR-AB ones (same value for pellet extract). At 24 h *post-mortem*, AB samples differed widely not only from GN ones but also from GR-AB, mainly for HIS extracts which contained most of the extractable myofibrillar proteins. By using values of extractabilities, AB samples could always be distinguishable from GN ones whatever the time *post-mortem* or the considered fraction. In contrast and for all the considered fractions, GR-AB samples were not always found to be different from the GN ones. Quantitative analysis of the presence of actin and myosin within the different HIS fractions by WB confirmed that the level of considered proteins was similar for all the samples at 20 min *post-mortem*. At 24 h *post-mortem*, there were always significantly smaller amounts of actin and MHC present in AB samples than in other ones, indicating a different degree of those proteins alteration between the different samples. Whereas these alterations of actin and MHC in fast glycolysing muscles have previously been reported by Rathgeber *et al.* (1999b), Alvarado and Sams (2004) reported no effect of *post-mortem* glycolysis acceleration on the extraction of myofibrillar proteins, as observed here, if AB samples are excluded from the analysis by comparing GR-AB with GN ones. This indicates that, depending on the presence or otherwise of AB samples among GR ones, the occurrence of myofibrillar protein alterations is in response to accelerated glycolysis *post-mortem*. The presence of an associated increase in muscle temperature only in AB samples, together with a lack of any difference in pHu values, suggests that AB samples must be considered as true PSE ones while GR-AB are probably less affected. The percentage of AB samples among all the GR ones could then explain why some authors found large effects on meat quality parameters due to accelerated *post-mortem* glycolysis, while others concluded that the combination low early *post-mortem* pH and high temperature values had a much smaller effect. It is generally accepted that the combination of low pH and high temperature values, within muscle, leads to protein alterations (Joo *et al.*,

1999; Wilson; van Laack, 1999) and consequently modifies protein extractabilities as well as meat quality (Alvarado and Sams, 2004; Gratacos-Cubarsi and Lametsch, 2008). To our knowledge, but with the exception of a report by Offer (1991) who estimated the part of myosin degraded in several pH and temperature conditions, neither the conditions nor the duration of pH and temperature limit values have precisely been described. As a consequence, we must ask what are the incorrect conditions for meat quality. For example, is a condition described by a pH value lower than 6.0 and a temperature lower than 40°C during 10 min worst than one described by a pH value of 6.2 and a temperature higher than 40°C during a shorter or a longer time? As muscle to meat transformation is a dynamic phenomenon depending on time, temperature and pH values, we could take into account these three parameters to better predict consequences on the future of proteins and on associated meat qualities.

All data collected to determine the energetic status of sampled muscles confirmed that AB as well as GR-AB had rapidly started *post-mortem* glycolysis. Indeed, GP level was the same in all samples, which is in good accordance with observed similar pH_u values because GP directly reflects the level of stock energy at the time of death (Monin and Sellier, 1985, Le Bihan-Duval *et al.*, 2008). At the end of the onset of *rigor mortis*, all the recorded values for energetic compounds were at the same level, indicating that the extent of *post-mortem* glycolysis was at the same level in all samples, (Warris *et al.*, 1988; El Rammouz *et al.*, 2004). This was in good agreement with final pH_u values. However, whereas glycogen and glucose levels were higher in GR (including AB and GR-AB samples) than in GN at 20 min *post-mortem*, the lactate content was the reverse. This demonstrated that glycolysis began more quickly in GR than in GN muscles, as could be deduced from pH_{20min} values (Shen *et al.*, 2006). In our study, lactate levels, which are mainly responsible for pH fall in *post-mortem* muscles, were similar in AB and GR-AB samples as were pH_{20min} values. PFK

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

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

between them (Molette *et al.*, 2002; Fraqueza *et al.*, 2006). In this study, we demonstrated that, depending on the origin of samples, AB or GR-AB group, one can find equal or higher L^* values for meat samples with a low initial pH value than in normal ones. If we take as a reference the cut-off value of $L^* > 52$, as proposed by Barbut (1997), we would sort all the AB samples as being PSE ones, while all the GR-AB would be considered as the GN ones, despite that they present very different early pH values. As a consequence, it seems clear that only measuring one early pH value is not sufficient to accurately detect samples liable to develop the meat quality alterations generally associated with PSE syndrome in poultry.

Conclusion

This paper demonstrates that a simple measurement of one early muscle pH value is probably insufficient to satisfactorily distinguish turkeys prone to develop PSE syndrome and all the associated consequences for the quality of meat. Indeed, if the presence of a high muscle temperature and a low pH value in muscle are required to develop PSE syndrome, the duration of the conditions encountered *post-mortem* are also fundamental. Further experiments describing the precise kinetics of pH and temperature evolution *post mortem* are necessary to adequately understand the etiology of the syndrome. The different possible combinations of (pH, temperature and duration) could have large consequences on muscle proteins and variably affect meat quality. Other investigations are also required to understand why, which and how some turkeys are able to develop such conditions of muscle to meat transformation leading to the poorest final quality of poultry products.

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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 $P < 0.05$.

Figure 3. Specific activities (in UI) of the PFK (PhosphoFructoKinase) and cytochrome oxydase c (COX) enzymes in the different groups of turkeys. Values are means $\pm$ SD. GN = Normal Glycolysing, GR = Fast Glycolysing, AB = Abnormal GR, GR-AB = GR birds 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 a different superscript differ significantly at $P < 0.05$.

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

^{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 $P < 0.05$.

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

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 $\pm$ 1.14 ^{b,A}	87.6 $\pm$ 4.68 ^B

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

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

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

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 post mortem		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.