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animal

Different rates of glycolysis affect glycolytic activities and protein properties in turkey breast muscle

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*Protein alterations of turkey breast muscles (*Pectoralis major*) were investigated at 20 min and 24 h* post mortem*. Specific activities, quantities and kinetic parameters of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and aldolase A were also determined at 20 min* post mortem*. Based on the pH values at 20 min* post mortem*, two groups of samples were classified as rapid glycolysis group (RG; $pH_{20\,min} = 5.80 \pm 0.07$,* n *= 20) and normal glycolysis group (NG; $pH_{20\,min} = 6.21 \pm 0.01$,* n *= 20). RG had lower specific activities of GAPDH and aldolase A than NG while V_m and K_m values of both enzymes were not different between groups. RG showed lower high ionic strength (HIS) and pellet protein extractabilities at 20 min* post mortem*. It also had lower low ionic strength (LIS) and HIS protein extractabilities at 24 h* post mortem*. Besides pellet protein, muscular protein extractabilities at 24 h* post mortem *were higher than at 20 min* post mortem*. From SDS-PAGE of samples at 24 h* post mortem*, RG exhibited lower band intensities at 45 and 200 kDa, which were further identified as actin and myosin heavy chain (MHC), respectively. Western blots revealed that relative amounts of actin and MHC at 20 min* post mortem *were not different between groups. However, RG muscles had less relative amount of actin at 24 h* post mortem*. It also indicated that amounts of actin and MHC increased with regard to* post mortem *time.*

Keywords: turkey breast muscle, aldolase A, GAPDH, actin, myosin heavy chain

Introduction

Pale, soft and exudative (PSE) meat characterizes meat which has a pale color, a soft texture and a poor water-holding capacity. In swine, an inherited impaired gene that causes animal sensitize to stress (porcine stress syndrome, PSS) is involved in PSE meat (Fujii *et al*., 1991; Mullen *et al*., 2006). Since PSE was considered as a key problem in the industry, many researches have been conducted in pork (for review see, Rosenvold and Andersen, 2003). More recently, it has also been mentioned in turkey and chicken (Barbut *et al*., 2005). In North America, over 5% to 30% of turkey breast muscles are detected as PSE-like meat (Barbut, 1993) and its incidence seems to increase (Chiang *et al*., 2004). However, in most of the studies carried out on poultry, PSE-like meats often showed lower ultimate pH than normal meat (Barbut, 1993; McKee and Sams, 1997). Even if the alterations of PSE turkey meat are very similar to those observed in pork, the environmental and/or genetic origin has not yet been proven in poultry (Chiang *et al*., 2004).

In the literature, PSE meat quality alterations are described to be mainly caused by the rapid rate of glycolysis in the early *post mortem* period, leading to the combination of a high temperature and a low pH value in muscles during the beginning of the *rigor mortis* onset (Choi *et al*., 2007). Such conditions are known to reduce protein functionality. Indeed, reduced sarcoplamic and myofibrillar protein solubilities in PSE meat have already been reported in both pork and turkey (pork: Boles *et al*., 1992; turkey: Rathgeber *et al*., 1999a; Molette *et al*., 2005).

Moreover, several studies focused on glycolytic enzymes to better understand why some pig muscles are more susceptible to an increased rate of *post mortem* pH (for review, see Bowker *et al*., 2000). In turkey, knowledge of glycolytic enzymes alteration in PSE-like meat is still lacking. Molette *et al*. (2005) recently found differences in SDS-PAGE patterns of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and aldolase A between fast glycolyzing and normal glycolyzing turkey breast muscles at 24 h *post mortem*. However, the authors could not conclude in the implication of these two enzymes in the increased rate of *post mortem* pH fall. The aim of the present study is to

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study the kinetic parameters of GAPDH and aldolase A in samples collected early *post mortem* from turkey breast muscles with different rates of glycolysis to finally determine whether these two glycolytic enzymes could really be used as markers to identify PSE-like turkey.

Material and methods

Animals

A flock of tom turkeys (*Meleagris gallopovo*), from a commercial grower (line B.U.T.9; BUT Ltd, Chester, UK), was bred until 19 weeks of age (average live weight = 15 kg). All the birds were fasten 24 h before slaughtering and then conventionally processed (Gastronome SA, Moncoutant, France). For each turkey, at the end of the slaughtering process, the pH value of breast muscle (*Pectoralis major*, Pm) was measured ($pH_{20\,min}$) following the method of Jeacocke (1977). Two grams of breast muscle was homogenized in 18 ml of 5 mM Iodoacetate using Ultra Turrax (IKA-WERKE, Staufen, Germany). The $pH_{20\,min}$ was then measured by using a portable pH-meter combined with a glass electrode. Twenty grams of muscle was also collected at 20 min *post mortem* and immediately frozen in liquid nitrogen.

From this flock, two groups of animals were selected according to their $pH_{20\,min}$ values: the first one (normal glycolysis, NG, $n = 20$) was made up with animals with a 'normal' $pH_{20\,min}$ value (6.21 ± 0.01) and the second one (rapid glycolysis, RG, $n = 20$) of birds with a low $pH_{20\,min}$ (5.80 ± 0.07). The inside temperature of the muscle was also measured at 20 min *post mortem*. Following a 4°C overnight storage, the ultimate pH-value (pHu) was directly measured by inserting a glass pH-probe into the Pm muscle. Samples were then collected as described above and kept at −80°C until analyzed.

Assays of aldolase A activity and its kinetic parameters

One hundred milligrams of samples collected at 20 min *post mortem* were homogenized with 20 ml of 100 mM phosphate buffer using Ultra Turrax. The homogenized sample was then sonicated for 2×1 min. The enzyme extract was obtained from centrifugation at $7500 \times$ **g**, 4°C for 10 min.

Aldolase A activity was spectrophotometrically measured following the method of Koeck *et al.* (2004) by using 12 M D-fructose-1,6-bisphosphate (Biochemika, Duisburg, Germany) as a substrate and 3.5 M hydrazine sulfate/1 mM EDTA, pH 7.5 as a detector of glyceraldehyde-3-phosphate formation. The reaction mixture contained 2 ml substrate, 1 ml detector and 0.1 ml of five times diluted enzyme extract. The activity was assayed over 15 min by a spectrophotometer (Lambda 20; Perkin-Elmer, Connecticut, USA) at 240 nm, 25°C. One unit is expressed as a change in absorbance of 1.00 per minute (unit/mg = $(\Delta A_{240\,sample} - \Delta A_{240\,blank})$/mg enzyme per ml reaction mixture). Maximum speed (V_m) and affinity to substrate (K_m) of aldolase A were determined by performing activity assays with various substrate, concentrations ranging from 3 mM up to 60 mM. According to Michaelis–Menten kinetics, plots of Lineweaver–Burk were made. V_m and K_m values were then calculated (Koeck *et al.*, 2004).

Assays of GAPDH activity and its kinetic parameters

The preparation of enzyme extract for GAPDH activity was performed as described above except that 125 mM of triethanolamine (TEA) was used as a buffer. This extract containing the enzyme was also used for western blot assays.

GAPDH activity was measured following the method described by Crow and Wittenberger (1979). The reaction mixture contained 125 mM TEA buffer, 1 mM sodium arsenate, 5 mM Cysteine-HCl, 1 mM NAD^+ and 4 mM glyceraldehyde-3-phosphate (Sigma, Seelze, Germany). The activity was assayed over 5 min at 340 nm, 30°C. Activity assays with different substrate's concentrations from 1 mM up to 20 mM were then performed to determine V_m and K_m values as previously described.

Protein extractability

Muscular proteins of Pm samples collected at 20 min and 24 h *post mortem* were extracted following Rathgeber and Boles' methods (Boles *et al.*, 1992; Rathgeber *et al.*, 1999a). One gram of sample was homogenized in 20 ml of a low ionic strength (LIS) buffer, containing 0.05 M potassium phosphate buffer, 1 mM NaN_3 and 2 mM EDTA/pH 7.3 at 2°C for 10 s. The homogenized sample was then agitated for 30 min at 4°C and centrifuged at $17\,500 \times$ **g**, 2°C for 15 min. Ten milliliters of the supernatant (*LIS fraction*) was collected at 2 cm above the bottom of the tube while the remaining supernatant was discarded. A supplemental 20 ml LIS buffer was added to resuspend the pellet, which was then homogenized, agitated and centrifuged as previously described. The supernatant was discarded and the procedure was repeated with high ionic strength (HIS) buffer, containing 0.55 M KCl, 0.05 M potassium phosphate buffer, 1 mM NaN_3 and 2 mM EDTA/pH 7.3, at 2°C. Following centrifugation, 10 ml of supernatant (*HIS fraction*) was collected as described above. The excess supernatant was removed. The pellet was resuspended in 40 ml of potassium phosphate buffer containing 75 mM KCl, 10 mM KH_2PO_4, 2 mM $MgCl_2$ and 2 mM EGTA/pH 7 and sonicated (Sonificateur®; Bioblock Scientific, Illkirch, France) for 1 min to solubilize 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 the total protein.

Sodium dodecyl sulfate-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*. It was also performed for enzyme extract from samples collected at 20 min *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, 1, 5 and 10 μg for western blots of GAPDH, actin and myosin heavy chain (MHC), respectively. Resolving gel of 12% polyacrylamide was used for LIS fraction and GAPDH enzyme extract 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.

Mass spectrometry

Proteins of interest were excised from Coomassie blue stained gels. Proteins were characterized after trypsin in-gel digestion, by peptide mass fingerprinting using a MALDI-TOF mass spectrometer (Voyager-DE™ STR; Perseptive Biosystems, Framingham, MA, USA), as previously described (Borderies *et al.*, 2003). Peptide mass fingerprints were compared to the database of all species and *Gallus gallus* from National Center for Biotechnology (NCBI) non-redundant database using MS-FIT (Protein Prospector; http://prospector.ucsf.edu). The searches were done with a tolerance of 20 p.p.m., considered oxidation of methionine and partial cleavage, leaving a maximum of one internal site uncleaved by trypsin. Identification was considered positive when the difference in the MOWSE score between the first and second ranked proteins was more than one order of magnitude.

Western blots of GAPDH, actin and myosin heavy chain

HIS fraction was used to perform western blots of actin and MHC. Enzyme extract was used to perform western blot of GAPDH.

Following performing SDS-PAGE, the gel was steeped into transfer buffer containing 25 mM Tris, 192 mM glycine and 20% v/v methanol (Towbin *et al.*, 1979) for 20 min. The proteins were transferred to nitrocellulose membrane (Hybond ECL; Amersham, Munich, Germany) at 30 V overnight using a transfer cell (EBU 4000; CBS scientific, California, USA). Membrane was blocked with milk buffer containing 3% w/v milk powder in phosphate buffer saline (PBS, pH 7.5) at room temperature (RT) for 1 h. Following blocking, the membrane was probed for specific proteins using primary antibody (mouse anti-GAPDH, MAB 374, chemicon, California, USA; mouse anti-actin, MAB 1501, chemicon; mouse anti-myosin, F59, DSHB, Iowa, USA) at RT for 2 h. The primary antibody-to-milk buffer ratio was 1 : 1000 for GAPDH and actin and 1 : 25 for MHC. Then, the membrane was washed three times with PBS and incubated in secondary antibody (goat anti-mouse IgG, AP 127, chemicon, California, USA) at RT for 1.5 h. The secondary antibody to milk buffer ratio was 1 : 50 000 for GAPDH and actin and 1 : 25 000 for MHC. Following this incubation, the membrane was washed three times and the chemiluminescent substrate (Super Signal® West Pico; PIERCE) was then used to detect the reactivity of the primary antibody with its antigen.

The photo of western blot was taken by making a contact between the membrane and a photo film (Amersham Hyperfilm™MP; GE Healthcare, Munich, Germany). The film was then developed by steep in 10% revelation solution (AL4; Kodak, New York, USA), distilled water and 10% fixation solution (LX24; Kodak) for 1 min each, respectively. The amount of 0.5 μg purified GAPDH was used as a reference amount for GAPDH while 2 and 5 μg proteins of chicken breast muscle were used as reference amounts for actin and MHC, respectively. The Band intensity of samples was then measured by using Image Analysis (ImageMaster 2D Platinum 6.0; GE Healthcare, New Jersey, USA). It was expressed as a percentage of reference protein (purified GAPDH, actin and MHC of chicken breast muscle).

Statistical analysis

All analyses were performed in duplicate. Results are expressed as the mean ± standard deviation (s.d.). Linear regression analysis was used to calculate V_m and K_m values. Only individual effect of group and effect of time were tested. Analyses of variance were performed using the GLM procedure of the Statistical Analysis Systems Institute (1989). Values showing $P < 0.05$ were considered significant.

Results

Animals and pH values

The $pH_{20\,min}$ value of the RG group was lower than that of the NG group. A difference of 0.5 pH unit was found as a result of the criterion used to select the animals. The inside temperature of muscle at 20 min *post mortem* were 42.1 ± 1.4 and 41.8 ± 0.7°C for RG and NG, respectively ($P > 0.05$). However, their pHu values were not different (5.80 ± 0.08 and 5.77 ± 0.09 for RG and NG, respectively; $P > 0.05$).

Specific activities of aldolase A and GAPDH and their kinetic parameters

The specific enzymatic activities of aldolase A and GAPDH and their kinetic parameters at 20 min *post mortem* are shown in Table 1. Both aldolase A- and GAPDH-specific activities of NG muscles were higher than those of RG ones. However, their V_m and K_m values for both enzymes were not different between the two groups.

Protein extractability

The muscular protein extractabilities of RG and NG samples at 20 min and 24 h *post mortem* are presented in Table 2. NG muscles had higher protein extractabilities in HIS and pellet fractions than RG ones at 20 min *post mortem*. NG muscles also had higher protein extractabilities in LIS and HIS fractions at 24 h *post mortem*.

At 20 min *post mortem*, protein extractabilities in LIS and HIS fractions, in both RG and NG muscles, were lower than those at 24 h *post mortem*. Moreover, the increase in

Table 1 *Effect of rates of glycolysis on specific activities and* V_m *and* K_m *values of aldolase A and GAPDH in turkey breast muscle collected at 20 min* post mortem

	Specific activity (unit/mg)	V_m (unit/mg)	K_m (mM)
Aldolase A			
RG	2.35 ± 0.21	1.90 ± 0.45	8.0 ± 2.2
NG	2.61 ± 0.28	1.93 ± 0.54	8.0 ± 2.9
P (group)	***	ns	ns
GAPDH			
RG	49.38 ± 19.02	15.3 ± 7.2	2.9 ± 1.4
NG	66.59 ± 24.71	19.9 ± 8.8	3.6 ± 1.7
P (group)	*	ns	ns

Values are given as mean ± s.d., $n = 20$.
RG = rapid glycolysis; NG = normal glycolysis.
$P > 0.05$ (ns); $^{*}P < 0.05$; $^{***}P < 0.001$.

Table 2 *Effect of rate of glycolysis and* post mortem *time on the muscular protein extractability in turkey breast muscle samples (expressed in percentage of total protein)*

	20 min	24 h	*P* (time)
	LIS fraction		
RG	24.81 ± 0.93	26.29 ± 2.05	**
NG	24.96 ± 1.03	29.28 ± 1.13	***
P (group)	ns	***	
	HIS fraction		
RG	6.79 ± 1.00	14.36 ± 6.00	***
NG	7.63 ± 1.17	18.16 ± 4.66	***
P (group)	*	*	
	Pellet fraction		
RG	26.31 ± 3.09	28.78 ± 6.07	ns
NG	30.70 ± 2.86	26.98 ± 4.36	**
P (group)	***	ns	

Values are given as mean ± s.d., $n = 19$ and 18 for RG and NG, respectively.
RG = rapid glycolysis; NG = normal glycolysis; LIS = low ionic strength; HIS = high ionic strength. $P > 0.05$ (ns); $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

protein extractability is higher for HIS (122% and 270% for RG and NG, respectively) than for LIS (6% and 17% for RG and NG, respectively) fractions. Protein extractability in pellet fraction of the RG group was not different between 20 min and 24 h *post mortem.* On the contrary, at 20 min *post mortem*, this fraction in the NG group had higher protein extractability than at 24 h *post mortem*.

SDS-PAGE and mass spectrometry

There were no detectable quantitative and qualitative differences in the banding pattern for protein in LIS fraction in relation to the rate of glycolysis and *post mortem* time (data not shown). Figure 1 shows the banding patterns of HIS fractions from RG and NG samples collected at 20 min and 24 h *post mortem*. Although the banding pattern at 20 min *post mortem* was similar between the two groups (Figure 1a), differences in band intensity were reported at 24 h *post mortem*. These differences were particularly noticeable at

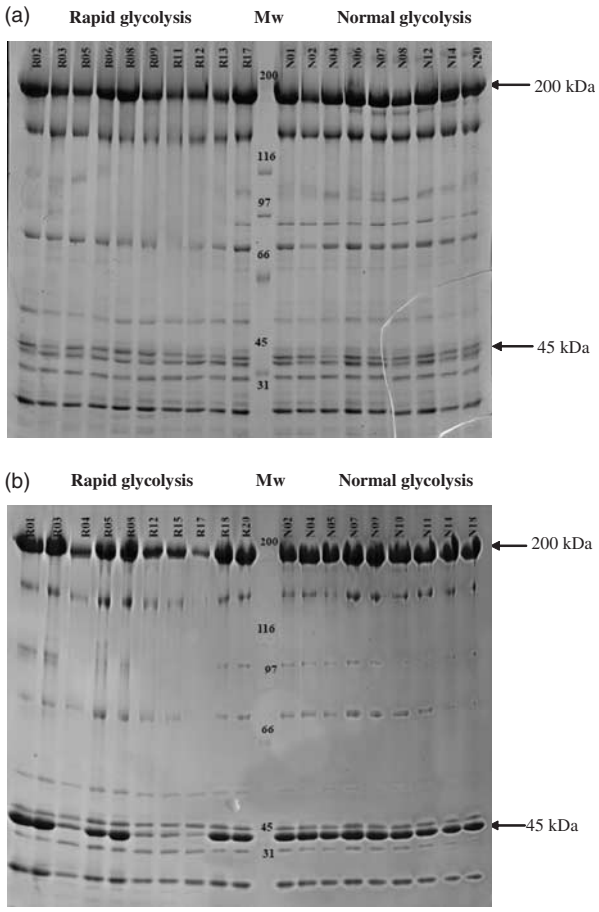

Figure 1 Gel electrophoresis patterns of HIS fraction from turkey breast muscles collected at 20 min (a) and 24 h (b) *post mortem*. Each lane contained 40 μg proteins and 8% polyacrylamide were used to prepare resolving gels. Gels were stained by Coomassie blue R-250. Mw = molecular weight in kDa.

45 and 200 kDa for four animals belonging to the RG group (Figure 1b). The general SDS-PAGE pattern of myofibrillar proteins is well known (Rathgeber *et al.*, 1999b) and the main bands (200 and 45 kDa) would likely correspond to MHC and actin. By mass spectrometry analysis, the identity of the proteins within these two bands was confirmed. Moreover, these four animals (group RG2) exhibited similar muscle pH at 20 min *post mortem* ($pH_{20\,min}$ 5.78 ± 0.12 and 5.81 ± 0.69 for RG2 and RG groups, respectively, $P > 0.05$) and muscular temperature (43.1 ± 2.2°C and 41.7 ± 0.9°C for RG2 and RG, respectively, $P > 0.05$). However, drip loss was lower in the RG2 group than in the RG group (6.2 ± 1.5% and 3.7 ± 1.1%, respectively, $P < 0.05$).

The amount of actin, MHC and GAPDH quantified by western blot

The image of western blotting of actin and MHC from RG and NG samples collected at 20 min and 24 h *post mortem* is shown in Figure 2. Amounts of actin and MHC increased with regard to *post mortem* time. At 20 min *post mortem*, amounts of actin and MHC were not different between the two groups. However, at 24 h *post mortem*, NG muscles had

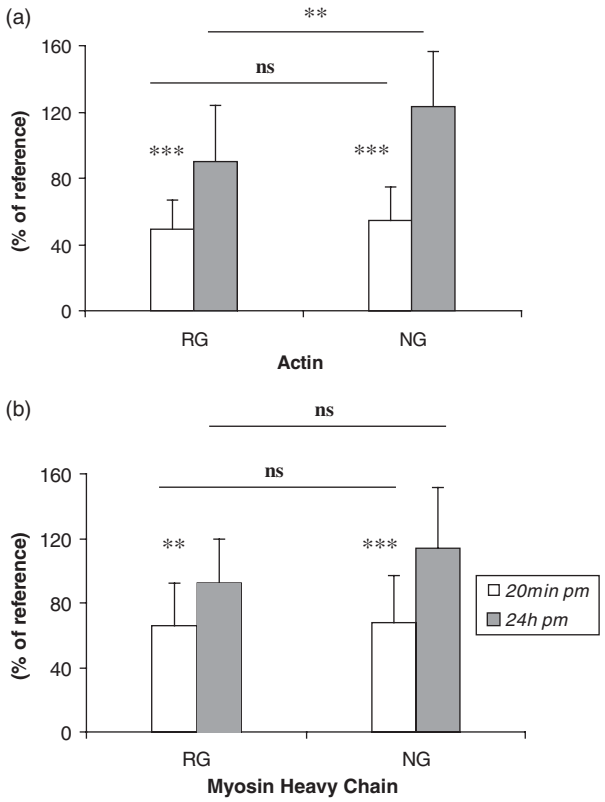

Figure 2 Relative amounts of actin (a) and myosin heavy chain (b) obtained using western blots from turkey breast muscles collected at 20 min and 24 h *post mortem* (mean ± s.d., $n = 19$ and 18 for RG and NG, respectively). Two and 5 μg proteins of chicken breast muscle were used as reference amounts for actin and MHC, respectively. RG = rapid glycolysis; NG = normal glycolysis. $P > 0.05$ (ns); $^{**}P < 0.01$; $^{***}P < 0.001$.

higher amount of actin than RG ones while no difference in the amount of MHC was reported (Figure 2b). Moreover, the four birds previously mentioned (RG2), exhibited a lower amount of MHC (64.4 ± 24.3% compared to 100.8 ± 21.2% for the other birds from RG group) and actin (54.4 ± 25.6% compared to 98.6 ± 30.4% for the other birds from the RG group).

Figure 3 shows the results of the amount of GAPDH quantified by western blot in samples collected at 20 min *post mortem*. The amount of GAPDH was not different between RG and NG groups.

Discussion

As already shown, breast meat from fast glycolysing turkeys is characterized by a pale color, a low water-holding capacity and cooking yield (Pietrzak *et al.*, 1997; Owens *et al.*, 2000). In the present study, the average value of $pH_{20\,min}$ of the RG group is similar to that of the low pH group studied in Pietrzak *et al.* (1997). In our study, birds in the RG group also had higher drip loss (4.2 ± 1.5% and 3.2 ± 0.8% for RG and NG, respectively, $P < 0.05$) and lower brine and cook yield than in NG ones (94.8 ± 4.4% and 98.3 ± 3.5% for RG and NG, respectively, $P < 0.001$).

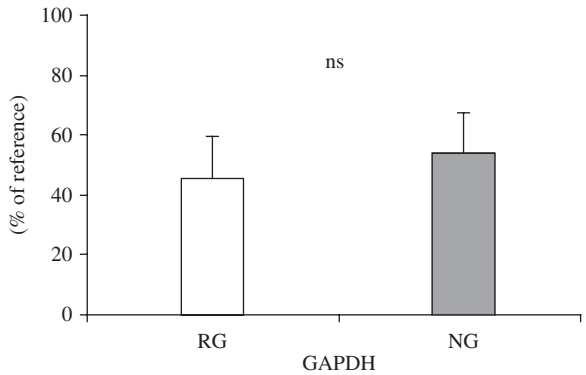

Figure 3 Relative amount of GAPDH obtained by using western blot from turkey breast muscle[1] collected at 20 min *post mortem* (mean ± s.d., $n = 20$). RG = rapid glycolysis; NG = normal glycolysis. $P > 0.05$ (ns).

Hereby, birds in the RG group met all these characteristics, and consequently can be defined as PSE-like meat while NG birds can be considered as normal or reference ones.

It is generally admitted that reduced *post mortem* pH values is caused by the rapid production and accumulation of lactic acid subsequent to the activation of the glycolysis pathway (Livisay *et al.*, 1996; Lyon and Buhr, 1999) with no evacuation from muscle due to the lack of blood circulation. Therefore to better understand why different rates of pH fall down could exist within same muscle, interest was taken into glycolytic enzyme activities. Several researches have been conducted on glycolytic enzymes properties, which could lead to PSE development (for review, see Scheffler and Gerrard, 2007). Among glycolytic enzymes, which account for about 70% of the sarcoplasmic protein content, GAPDH and aldolase A are found in large quantities (Kijowski, 2001). Moreover, qualitative differences were recently mentioned between PSE-like and normal turkey meat (Molette *et al.*, 2005). The specific activities of both aldolase A and GAPDH were higher in RG muscles than in NG ones. The findings were unexpected because RG muscles represented samples with a rapid rate of glycolysis (due to a rapid rate of pH decline), and consequently glycolytic enzyme activities or/and quantities could be supposed to be higher than in NG ones. However, we cannot exclude the fact that these enzymes might be altered by the combination of low pH and high muscle temperature values. Even if measured temperatures at 20 min *post mortem* were not different between RG and NG groups, the duration of unfavorable muscle conditions could be longer in RG muscles, leading to a higher protein denaturation (Offer, 1991). Therefore, such conditions could lead to a loss in protein functionalities and/or enzyme-specific activity. Several studies reported that most glycolytic enzymes bind together with muscle actin and MHC to provide glycolytic multi-enzyme complexes (GMEC). These GMEC have particular kinetic properties and high abilities to interact with other enzymes or the actin structure as well (MacGregor *et al.*, 1980; O'Reilly and Clarke, 1993; Rakus *et al.*, 2003; Dziewulska-Szwajkowska *et al.*, 2004). Therefore, the

different specific activities of aldolase A and GAPDH between RG and NG could be a result of a different complex formation in the muscle.

Kinetic parameters (i.e. K_m and V_m) were determined and no significant differences were pointed out between the two groups. To our knowledge, it is the first time that these parameters are evaluated in turkey muscle. The Michealis–Menten constant (K_m) reflects enzyme affinity for a given substrate and is specific, for a given enzyme, in a tissue and a species but vary within a wide range for different species or tissues (http://www.brenda-enzymes.info). However, within a species or a tissue, different K_m values can be found according to the primary sequence of the enzyme (Maurady *et al.*, 2002). In our study, one of the hypothesis was that the increased rate of *post mortem* pH fall might be explained by a different enzyme structure and consequently, different kinetic parameters. Unfortunately, this possibility was infirm.

Muscular protein characteristics were also studied with regard to their extractabilities at 20 min and 24 h *post mortem*. RG birds presented lower protein extractabilities than NG ones both at 20 min (in HIS and pellet fractions) and at 24 h *post mortem* (in LIS and HIS fractions). Reduced protein extractability in RG could be issued from a combination of a low muscle pH and a high carcass temperature values, which changed protein structure, leading to a modification of solubility (Gil *et al.*, 1998). Nevertheless, Ma and Addis (1973) could not notice any different extractabilities of sarcoplasmic and myofibrillar fractions in turkey breast muscle with various rates of *post mortem* glycolysis. On the contrary, Rathgeber *et al.* (1999a) found lower extractabilities of LIS and HIS fractions in PSE turkey muscle at 15 min *post mortem*. The present results reported at 24 h *post mortem* were also in agreement with those of Rathgeber *et al.* (1999a). Boles *et al.* (1992), Warner *et al.* (1997) and Joo *et al.* (1999) also found similar results in PSE meat in pork.

In the present study, besides pellet fraction, muscular proteins at 24 h *post mortem* showed higher extractability than at 20 min *post mortem*. These higher extractabilities at 24 h *post mortem* could result from meat aging when endogenous enzymes, particularly proteinases, caused alterations of muscle fibers (Kijowski, 2001). Indeed, Gil *et al.* (1998) observed a large increase in myofibrillar protein extractability after 14 days of *post mortem* storage in pork. On the contrary, in pork, Ryu *et al.* (2005) found a reduction of sarcoplasmic and myofibrillar protein extractabilities during *post mortem* storage. In the present study, higher extractabilities at 24 h *post mortem* could be the result of degradations of actin and MHC during the *post mortem* period (Lametsch *et al.*, 2003), which consequently showed higher band intensities from SDS-PAGE and higher relative amount from western blot assays.

Because extractability measurements are only representative of the protein content in each fraction, the qualitative pattern of proteins in both RG and NG at 20 min and 24 h *post mortem* was better distinguished by performing SDS-PAGE. At 20 min *post mortem*, from SDS-PAGE, no differences in protein patterns were noticeable. On the contrary, at 24 h *post mortem*, heterogeneity in band intensity in the HIS fraction for RG birds is clearly noticeable. These lower SDS-PAGE band intensities of actin and MHC in RG at 24 h *post mortem* could imply that, among this group, the response of muscle protein to the increased rate of *post mortem* pH decline could differ. Our findings were in agreement with those of Rathgeber *et al.* (1999b) who indicated that among sarcoplasmic and myofibrillar proteins, myosin is the major protein influenced by different rates of glycolysis. Pietrzak *et al.* (1997) also found less MHC solubility in PSE turkey breast muscle than that in normal muscle. Lovell *et al.* (1981) and Offer (1991) suggested that lower extractability is a result of a tighter combination of actin and MHC in PSE pork muscle than in normal one. It could be also applied to turkey breast muscle in this study because results obtained from western blot assays showed that RG muscles had a lower amount of actin at 24 h *post mortem*.

Conclusion

Results of this study indicate that the rate of glycolysis and *post mortem* time affect turkey breast muscle, leading to changes in protein extractability. At 20 min *post mortem*, no particular differences in protein band patterns and amounts of actin and MHC between samples from different rates of glycolysis were found, which implies that it might be too early to investigate protein alteration at that time. Findings also indicate that actin and MHC are degraded with regard to *post mortem* period and that actin is the major protein affected by rate of glycolysis at 24 h *post mortem*. The results of the enzymatic kinetic parameters of aldolase A and GAPDH at 20 min *post mortem* imply that considering only these two enzymes are not sufficient to classify PSE meat from normal one. Their activities were also modified by the lower $pH_{20\,min}$ probably due to the enzyme alteration caused by the combination of low pH and high muscle temperature early *post mortem*.

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