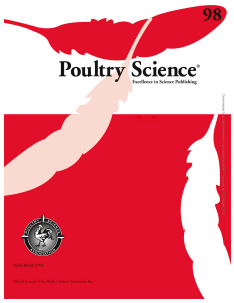

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The role of histidine dipeptides on post-mortem acidification of broiler muscles with different energy metabolism

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ABSTRACT

It is generally held that the content of several free amino acids and dipeptides is closely related to the energy-supplying metabolism of skeletal muscles. Metabolic characteristics of muscles are involved in the variability of meat quality due to their ability to influence the patterns of energy metabolism not only in living animal but also during post-mortem time. Within this context, this study aimed at establishing whether the concentration of histidine dipeptides can affect muscle post-mortem metabolism, examining the glycolytic pathway of three chicken muscles (*Pectoralis major*, *extensor iliotibialis lateralis* and *gastrocnemius internus* as glycolytic, intermediate and oxidative-type, respectively) selected based on their histidine dipeptides content and ultimate pH. Thus, a total of 8 carcasses were obtained from the same flock of broiler chickens (Ross 308 strain, females, 49 days of age, 2.8 kg body weight at slaughter) and selected immediately after evisceration from the line of a commercial processing plant. Meat samples of about 1 cm^3 were excised from bone-in muscles at 15, 60, 120 and 1,440 min post-mortem, instantly frozen in liquid nitrogen and used for the determination of pH, glycolytic metabolites, buffering capacity as well as histidine dipeptides content through ^{1}H-NMR. Overall results suggest that glycolysis in leg muscles ceased already after 2 h post-mortem, while in breast muscle continued until 24 h, when it exhibited significantly lower pH values ($P<0.05$). However, considering its remarkable glycolytic potential, *Pectoralis major* muscle should have exhibited a greater and faster acidification, suggesting that its higher ($P<0.05$) histidine dipeptides' content might have prevented a potentially stronger acidification process. Accordingly, breast muscle also showed greater ($P<0.05$) buffering ability in the pH range 6.0-7.0. Therefore, anserine and carnosine, being highly positively correlated with muscle's buffering capacity ($P<0.001$), might play a role in regulating post-mortem pH decline, thus exerting an effect on muscle metabolism during pre-rigor phase and the quality of the forthcoming meat. Overall results also suggest that total histidine dipeptides content along with muscular ultimate pH represent good indicators for the energy-supplying metabolism of chicken muscles.

Key words: histidine dipeptides; broiler; post-mortem metabolism; glycolysis; buffering capacity.

INTRODUCTION

Skeletal muscles have to withstand a large range of activities, from supporting the body weight during periods of standing, to perform rapid movements following sudden threats. To deal with a huge variety of activities, muscles are composed by various types of fibers, which differ in their contractility, metabolic activity as well physiological, morphological and other distinctive characteristics (Ryu and Kim, 2005; Westerblad et al., 2010; Lee et al., 2010). Two major metabolic pathways are used to produce energy (i.e. ATP) in skeletal muscles: the first is the oxidative pathway, through which carbohydrates, lipids and amino acids are oxidized in the mitochondria with a high oxygen requirement, while the second is the glycolytic pathway, through which glycogen stores are rapidly converted into lactate without any oxygen requirement (Scheffler and Gerrard, 2007; Aberle et al., 2012). These two metabolic pathways have been used to generally type myofibers as oxidative, glycolytic or oxido-glycolytic (i.e. intermediate). According to their fiber composition, muscles possess different abilities to release and seize Ca^{2+}, activate ATPases, stimulate glycolysis, produce lactate, and decrease post-mortem muscular pH (Lefaucheur, 2010; Zhang et al., 2017). Both in mammals and birds, metabolic characteristics of skeletal muscles are one of the focal factors associated to the variability of meat quality due to their ability to influence the pattern of energy-supplying metabolism in living animal, as well as during the conversion of muscle to meat occurring during post-mortem time (Lee et al., 2016; Petracci et al., 2017; Chauhan and England, 2018). In the past decades, several authors have suggested myoglobin concentration and lactate dehydrogenase activity to rapidly distinguish the oxidative or glycolytic muscle's patterns of energy generation, respectively (Flores et al., 1996; Hernández et al., 1998). More recently, based on the assumption that the content of several dipeptides and free amino acids is tightly linked to the muscle's metabolic type (Cornet and Bousset, 1999), Mora et al. (2008) have proposed carnosine content as a good indicator of muscle glycolytic metabolism, since it has been widely reported that its muscular concentration increases with the glycolytic activity of the muscle (Boldyrev and Severin, 1990; Aristoy and Toldra, 1991; Intarapichet and Maikhunthod, 2005).

Carnosine (β-alanyl-L-histidine), along with anserine (β-alanyl-l-N-methylhistidine), are histidine-containing dipeptides widely abundant in skeletal muscles of mammals and other vertebrates, exploiting several biological functions (Barbaresi et al., 2019). Their amount greatly varies depending on the specie and the muscle considered (Gil-Agustí et al., 2008). However, since poultry meat is particularly rich of histidine-containing dipeptides (Tinbergen and Slump, 1976), both carnosine and anserine have been the object of several poultry science-based studies because of their biological importance (Kai et al., 2015; Kim et al., 2018; Barbaresi et al., 2019). Indeed, being highly involved in the homeostasis of muscles, a reduction of their concentrations has been recently found to be associated with the occurrence of emerging muscle abnormalities in chickens (Sundekilde et al., 2017; Soglia et al., 2019; Baldi et al., 2020a). These compounds act as metal ion chelators, free radical scavengers and natural buffers to contrast the acidic end-products (e.g. lactic acid and hydrogen ions) generated during the anaerobic metabolism in vivo, since their pK_a is close to the physiological pH of animal tissues (Castellini and Somero, 1981; Decker, 2001; Wu et al., 2003). It is believed that, as in vivo, also during post-mortem anserine and carnosine regulate muscular pH (Puolanne and Kivikari, 2000). With this in mind, it is reasonable to hypothesize that the muscular concentration of histidine dipeptides might provide a sort of resistance to pH drop after the death of the animal, thus having consequences on muscle metabolism during the pre-rigor phase. Within this scenario, the main objective of the study was establishing the relation between the content of histidine dipeptides and muscle post-mortem metabolism, examining the metabolic pathways of chicken muscles selected on the basis of their amount of anserine and carnosine to represent the main metabolic types (glycolytic, intermediate and oxidative).

MATERIALS AND METHODS

Muscle Sampling

For the purpose of the study, three different chicken muscles were needed to represent the main energy-yielding patterns (oxidative, glycolytic and intermediate), in order to investigate the relation between the amount of histidine-containing compounds and muscle post-mortem metabolism.

Muscles needed to meet the following criteria: first, they must be supposedly characterized by a different in vivo energy metabolism, they must be of interest for human consumption, and, lastly, be readily available for easy sampling post-mortem. Thus, considering that both the amount of histidine dipeptides and the pH_u of a muscle are somehow related to its energy-generating pathway (Mora et al., 2008; Westerblad et al., 2010) a preliminary study has been carried out in order to select three muscles chosen on the basis of both their histidine dipeptides content and pH_u to represent the best compromise among the aforementioned criteria (see supplementary material S1). On a batch of ten chicken muscles belonging to different anatomical regions, those selected for the experiment to represent the three main metabolic types were: *Pectoralis major* (PM; breast) as the glycolytic-type muscle (pH_u: 5.84; histidine dipeptides: 521.9 mg/100g meat); *extensor iliotibialis lateralis* (EIL; thigh) as the intermediate-type muscle (pH_u: 6.38; histidine dipeptides: 269.3 mg/100g meat) and *gastrocnemius internus* (GI; drumstick) chosen to represent a predominantly oxidative-type of muscle (pH_u: 6.57; histidine dipeptides: 196.2 mg/100g meat) (Figure 1).

A total of eight carcasses were obtained from the same flock of broiler chickens (Ross 308 strain, females, 49 days of age, 2.8 kg body weight at slaughter) farmed and harvested under standard commercial conditions. Before slaughter, animals were subjected to a total feed withdrawal of 8 h, including a 2 h lairage time at the processing plant. Birds were electrically stunned (150 mA/bird, 400 Hz), killed by severing the jugular vein and carotid artery with an automatic device and bled for 180 s. Subsequently, birds were scalded 51 to 52°C for 215 s, plucked and eviscerated. Carcasses were selected immediately after evisceration from the line of the processing plant and meat samples of about 1 cm^3 were excised from bone-in PM, EIL and GI muscles at 15, 60, 120 and 1,440 min post-mortem, instantly frozen in liquid nitrogen and stored at -80°C until analyses. Carcasses were stored at 4 ± 1°C for the whole duration of the trial and muscle internal temperature was monitored in the cranial part of the left *Pectoralis major* muscle through a digital temperature thermal probe sensor (Hanna Instruments, Italy). Birds were housed, handled, transported from farm to

slaughterhouse and slaughtered according to the principles stated in EU Legislation regarding the protection of farmed animals (European Commission, 2005, 2007, 2009).

pH Measurements and Metabolite Analysis

Samples were processed as described by Matarneh et al. (2018) with slight modifications. Briefly, frozen meat samples (n=8/muscle/sampling time) were powdered under liquid nitrogen using a mortar and pestle. For pH analysis, powdered samples (0.1 g) were homogenized for 3 min using a Multi-Vortexer (Thomas Scientific, USA) in 0.8 mL of ice-cold 5 mM sodium iodoacetate and 150 mM KCl solution (pH = 7.0). Following centrifugation at 17,000 × g for 5 min and equilibration to 25°C, pH of supernatants was directly measured using a pH glass electrode (Jenway, UK). Aliquots of 0.1 g of frozen powdered samples designated for glucose, glucose-6-phosphate (G6P) and lactate analysis were homogenized for 3 min using a Multi-Vortexer (Thomas Scientific, USA) in 1 mL of ice-cold 0.5 M perchloric acid and incubated on ice for 20 min. Homogenates were centrifuged at 17,000 × g for 5 min, then supernatants were transferred into new tubes and neutralized with 2M KOH. As for muscle glycogen analysis, another aliquot of powdered sample was homogenized for 3 min using a Multi-Vortexer (Thomas Scientific, USA) in 1 mL of 1.25M HCl, heated at 90°C for 2 h and centrifuged at 17,000 × g for 5 min. Supernatants were transferred into new tubes and neutralized with 1.25M KOH. Glycogen, glucose, G6P and lactate concentrations (expressed as µmol/g) were determined using enzymatic methods modified for a 96-well plate as described by Hammelman et al. (2003). In addition, glycolytic potential (GP) was calculated following the equation: GP (µmol lactate/g muscle) = 2 * (glucose + G6P + glycogen) + lactate, as proposed by Scheffler et al. (2013).

Buffering Capacity

Buffering capacity of meat samples was determined according to the method proposed by Matarneh et al. (2015) with slight modifications. About 2.5 g of the 1,440min post-mortem meat (n=8/muscle) was homogenized with an Ultra-Turrax T-25 (IKA-Werke, Germany) in 25 ml of ice-cold 5mM sodium iodoacetate and 150 mM KCl solution (pH = 7.0). After equilibration to 25°C, the

homogenate was transferred into a beaker and the initial pH (pH_i) was measured while stirring. The pH of homogenate was adjusted to 6.0 by adding HCl or NaOH and then titrated to 7.0 using 0.5 M NaOH. Samples pH was measured using a pH glass electrode (Jenway, UK) and buffering capacity was calculated as follows: buffering capacity = ΔB / ΔpH, where ΔB is the increment of base expressed as µmol NaOH/g of tissue and ΔpH is the corresponding pH variation following the addition of NaOH.

Histidine Dipeptides

The concentration of anserine and carnosine in chicken meat samples was assessed through proton nuclear magnetic resonance spectroscopy (^{1}H-NMR), as previously described by Marcolini et al. (2015) with slight modifications. Briefly, about 0.5 g of the 1,440 min post-mortem meat (n=8/muscle) were homogenized in 3 ml of distilled water by Ultra-Turrax T25 basic (IKA-Werke, Germany) (20 s at 11,000 rpm). Then, 1 ml of homogenate was transferred into a new tube and centrifuged at 14,000 rpm for 10 min at 4°C. An aliquot (700 µL) of supernatant was added into a new tube with 800 µL of chloroform, vortexed and centrifuged as before. Subsequently, 500 µL of the supernatant were added to 200 µL of potassium phosphate buffer (1M, 2mM sodium azide; pH 7.0) in D_2O and 10 mM 3-(Trimethylsilyl) propionic-2,2,3,3-d4 acid sodium salt (TSP). Samples were centrifuged at 14,000 rpm for 10 min and 700 µL of the supernatant were transferred into NMR tube. ^{1}H-NMR spectra were then recorded at 25°C with a Bruker Avance III spectrometer operating at 600 MHz, equipped with a BBI-z probe and a B-ACS 60 sampler for automation (Bruker BioSpin, Germany). Spectra were collected with a 90° pulse of 14 µs with a power of 10 W, a relaxation delay of 5 s, and an acquisition time of 2.28 s.

Statistical Analysis

Data concerning pH and glycolytic metabolites were analyzed using the ANOVA for repeated measurements by employing the GLM procedure of SAS software (SAS Institute Inc., USA), testing the effect of the sampling time (15, 60, 120 and 1,440 min). The same dataset was also processed with the one-way ANOVA to test the main effect of the muscle type (PM-glycolytic,

EIL-intermediate and GI-oxidative) on pH and glycolytic metabolites for each sampling time. Data concerning buffering capacity and histidine dipeptides were analyzed using one-way ANOVA, considering the muscle type as a main effect. Differences among mean values were then investigated by Tukey's HSD test, by considering a significance level of $P<0.05$. Furthermore, to investigate the relationship between histidine dipeptides concentration, buffering capacity and muscles glycolytic potential, correlation coefficients between the variables were generated using the Pearson's correlation option present in SAS software (SAS Institute Inc., USA).

RESULTS AND DISCUSSION

pH decline

The rate and the extent of muscular acidification occurring post-mortem can exert profound effects on meat quality and depend on several aspects, such as environmental factors, the specie considered, the physiological state of the muscle as well as its energy-supplying metabolism (Eskin et al., 2013; Lonergan et al., 2019). The patterns of pH decline of selected muscles during post-mortem time are shown in Figure 2. Intriguingly, within the first 120 min post-mortem, PM and GI muscles showed the same acidification onset, while EIL outpaced showing significantly lower pH values in the same post-mortem time frame ($P<0.05$). However, both GI and EIL muscles did not show any further significant decrease in pH value between 120 and 1,440 min post-mortem, meaning that the acidification process of these muscles reached a plateau already at 2 h post-mortem, while PM muscle's pH continued to drop until 24 h post-mortem. Indeed, at 1,440 min, PM exhibited significantly lower pH values if compared to both thigh and drumstick muscles (5.88 vs. 6.12 and 6.35, respectively; $P<0.05$). The overall extent of muscle acidification was greatly different between muscles, with PM showing a ΔpH of 1.02 units, EIL of 0.37 and, lastly, GI muscle of 0.26. These divergences in the acidification extent are ascribable to several factors, among which we found the different type of fibers composing the muscles themselves and, consequently, the amount of substrates available at death to enter into the glycolytic pathway (i.e. glycolytic potential) (Pearson and Young, 1989; Schreurs, 2000; Young et al., 2004; Pösö and

Puolanne, 2005). The majority of skeletal muscles are composed by a mixture of fiber types (Pollard et al., 2017). It is generally held that locomotor muscle, designated for low intensity exercise, are mainly made up by a combination of type I and IIa fibers (i.e. oxidative and intermediate, respectively) in most farmed animals (Valberg, 2008; Zhang et al., 2017). On the contrary, muscle that must withstand maximal exercise intensity are mainly composed by glycolytic fibers, such in the case of *Pectoralis major* in broilers (Schreurs, 2000; Branciari et al., 2009). Thus, from an energy metabolism perspective, glycolytic muscles such as chicken breast usually exhibit higher glycolytic potential and contraction speed that lead to great and fast acidification patterns post-mortem, while leg muscles usually display slow acidification rates and pH_u values higher than 6.0 (Valberg, 2008; Petracci et al., 2017). Having this in mind, PM should have exhibited a faster and greater pH decline, especially in the first 2 h post-mortem when, unexpectedly, PM and GI showed an analogous acidification process despite their different in vivo energy-yielding pathways.

Glycolytic metabolites

For better understanding of post-mortem metabolism and tracking the progression of anaerobic glycolysis, the concentrations of glycolytic metabolites were measured in chicken PM, EIL and GI muscles (Figure 3). Patterns of lactate formation followed pH decline and confirmed the differences in both acidification rate and extent detected among the muscles of different energy-yielding metabolism (Figure 3a). Accordingly to pH results, at 24 h post-mortem PM showed significantly higher ($P<0.05$) lactate concentrations if compared to both leg muscles, in agreement with what previously found by Berri et al. (2005). However, it is noteworthy to highlight that, considering the average lactate levels detected in PM at 15 min post-mortem (19 µmol/g), breast muscle should have exhibited a lower pH at the same time point. Indeed, EIL muscle, showing analogous lactate concentration (18.9 µmol/g), exhibited a significantly ($P<0.05$) lower pH at 15 min if compared to breast (6.49 vs. 6.62, respectively; Figure 2).

Mobilization of muscle glycogen during post-mortem glycolysis likely drives pH decline and might provide useful information concerning substrate utilization in muscles of different energy-supplying

metabolism (Matarneh et al., 2018). Patterns of glycogen depletion during post-mortem time are shown in Figure 3b. If compared to thigh and drumstick, breast muscles showed significantly higher content of glycogen at 15, 60 and 120 min post-mortem ($P<0.05$) and the fastest glycogen depletion rates (i.e. greater glycogenolytic activities) confirming what observed by Villa Moruzzi et al. (1981) in glycolytic and oxidative muscles from rats. Fast-twitch, glycolytic fibers generally have great glycogen storages since they need to quickly take it up to sustain brief and intense movements (i.e. wing flapping in flightless birds such as chickens and turkeys), while slow-twitch, oxidative fibers are highly efficient in ATP synthesis, thus needing less glycogen and glucose to provide energy through glycolysis (Schreurs, 2000; Shen et al., 2015; Zhang et al., 2017). Accordingly, chicken PM possessed a greater carbohydrate flux entering the post-mortem glycolysis, justifying the significantly lower ultimate pH and higher lactate concentration at 1,440 min post-mortem if compared to leg muscles (see Figure 2 and 3a, respectively). Glycogen was almost depleted within 120 min post-mortem in GI and EIL muscles, which did not show any further decrease between 2 and 24 h post-mortem, corroborating the achievement of their pH_u (i.e. cessation of post-mortem glycolysis) after 2 hours from the death of the animal. On the contrary, glycogenolysis proceeded in PM muscle until 1,440 min post-mortem, where residual glycogen (2.30 µmol/g) found in meat samples suggest that glycolysis could have further continued. Indeed, glycogen is not usually a glycolysis rate-limiting factor in chicken breast muscles (Baldi et al., 2020b).

Glycogen degradation yields non-phosphorylated glucose molecules and glucose 1-phosphate, which is isomerized to G6P and enters the glycolytic pathway, while free glucose molecules are either converted by hexokinase to G6P or accumulated in post-mortem muscle (England et al., 2017; Matarneh et al., 2018). Patterns of glucose utilization and G6P generation (figure3c and 3d, respectively) reflect the balance between glycogen depletion and lactate production as glycolysis proceeds (Aliani et al., 2013). At 15 min post-mortem, GI muscles showed significantly lower glucose and G6P concentrations ($P<0.05$), supporting once again the reduced flux of substrates entering the post-mortem glycolysis that led to higher pH_u values. As previously found for cattle

(Koutsidis et al., 2008) and chicken muscles (Matarneh et al., 2018), a reduction in both glucose and G6P concentrations was observed in the first hours post-mortem. While from 120 min post-mortem onwards glucose levels in both EIL and GI remain stable ($P>0.05$), PM muscles showed a significant increase in glucose concentration, showing the highest values at 1,440 min post-mortem. This remarkable build-up of glucose in PM muscle from 120 min post-mortem onwards might be explained with a possible expanded activity of glucose 6-phosphatase, an enzyme that hydrolyzes G6P into free glucose and a phosphate group (Van Schaftingen and Gerin, 2002). Albeit few information is available for avian species, the activity of this enzyme was found to be increased in glycolytic rather that oxidative fibers of mice during early post-mortem period (Watanabe et al., 1986). Apart from the muscle type, from 120 min post-mortem onwards G6P accumulates in the muscles thus corroborating what has been previously found for porcine, cattle and chicken muscles (England et al., 2014; Scheffler et al., 2015; Matarneh et al., 2018). Intriguingly, overall reduced G6P concentrations detected in GI muscle during post-mortem might suggest that G6P is generated at a rate comparable to its consumption, since hexokinase (i.e. the enzyme that catalyzes the conversion of glucose into G6P) activity is greater in muscle mainly composed by oxidative fibers (Lefaucheur, 2010).

Muscle glycolytic metabolites can be combined into a single measure termed as glycolytic potential, a sum of all the compounds that can be potentially converted into lactate, useful to indicate the muscle's capacity to extend post-mortem glycolysis (Monin and Sellier, 1985; Laack et al., 2001; Scheffler and Gerrard, 2007). As shown in Figure 4, the type of muscle significantly affected the glycolytic potential. Breast muscle showed significantly higher ($P<0.05$) glycolytic potential rather than leg muscles, among which GI showed the lowest value (35.3 µmol lactate/g). In more detail, glycolytic potential was found to be 2-fold higher in PM if compared to GI, while EIL muscle showed intermediate values. In the living animal, glycolytic potential is closely related to the myosin heavy chain isoforms expressed by the muscle fiber types, i.e. to their speed contraction (Shen et al., 2015). Fast-twitch fibers are characterized by a higher rate of ATP

consumption as well as a greater glycolytic potential than slow-twitch ones (Zhang et al., 2017). As a consequence, a higher glycolytic potential will lead to a greater production of lactate and the achievement of a lower ultimate pH (Berri et al., 2005; Choe et al., 2008), such in the case of PM muscle. In this regard, the strong relationship between glycolytic potential, meat pH_u and muscle metabolism has been widely proved (Monin et al., 1987; Berri et al., 2005). Thus, glycolytic potential outcomes further support our initial hypothesis that PM, EIL and GI muscles, chosen on the basis of their histidine dipeptides content, are presumably characterized by a different in vivo energy-supplying metabolism.

Buffering capacity

The onset of post-mortem metabolism can also be affected by muscle buffering capacity, that is the ability of intracellular fluids to buffer the acidic end-products formed during periods of anaerobic metabolism (Castellini and Somero, 1981). The majority of biological tissues is adapted to operate at pH near to 7.0. In vivo, if skeletal muscle has no buffers, the simultaneous production of lactate and protons during short-term bursts of anaerobic glycolysis will result in a fast pH drop, that may inhibit the effective function of some regulatory and vital enzymes (Hand and Somero, 1982; Robergs et al., 2004). As a general rule, buffering capacity is higher in muscle mainly composed by fast-twitch, glycolytic fibers since in vivo they generate ATP through anaerobic glycolysis by producing great amounts of lactate, and for this reason they are accustomed to prevent excessive drops in pH (Pösö and Puolanne, 2005). Accordingly, in the pH range of 6.0-7.0, PM exhibited significantly higher ($P<0.05$) buffering capacity values compared to leg muscles, among which GI showed the lowest ones (Figure 5). It is widely reported that the buffering ability of a muscle is due by half to myofibrillar proteins, while compounds such as lactate, phosphate as well as histidine dipeptides contributed to the other half (Matarneh et al., 2017). Since poultry meat is known to possess high amounts of histidine-containing compounds (Barbaresi et al., 2019), the variations in buffering capacity between selected chicken muscles might be ascribable to the concentration of histidine dipeptides, which are believed to be accountable for the differences in buffering capacity

311 both within and between animal species (Castellini and Somero, 1981; Rao and Gault, 1989;
312 Decker, 2001; Jung et al., 2013). It is essential to mention that the distribution of histidine
313 dipeptides is species specific. Indeed, anserine was found to be plentiful in lamb and chicken meat
314 but scarce in beef, pork, and turkey, that in turn usually exhibit higher amounts of carnosine (Chan
315 and Decker, 1994). Therefore, while carnosine could be the major discriminating factor for
316 dissimilarities in buffering capacity among porcine and bovine muscles, anserine can help to better
317 explain differences detected within chicken meats.

318 ***Histidine dipeptides***

319 The concentration of anserine and carnosine in chicken meat greatly varies depending on the breed,
320 the gender, the age of the animals as well as the muscle considered (Peiretti and Meineri, 2015;
321 Barbaresi et al., 2019; Cheol Kim et al., 2020). In agreement with what previously observed by
322 several authors (Chan and Decker, 1994; Barbaresi et al., 2019), beside the muscle type, chicken
323 meat was found to be characterized by higher amounts of anserine rather than carnosine (Figure 6).
324 Furthermore, the concentration of histidine-containing dipeptides significantly differed depending
325 on the energy-supplying metabolism of muscles, confirming the outcomes of previous studies
326 (Intarapichet and Maikhunthod, 2005; Jung et al., 2013). PM muscle, being totally composed by
327 fast-twitch, glycolytic fibers (Branciari et al., 2009), accordingly showed the highest amount of both
328 anserine and carnosine, which resulted to be correspondingly 3.4- and 3.0-fold higher than GI
329 muscles (409.0 vs 118.1 and 136.5 vs 45.6 mg/g meat, respectively; $P<0.05$), that in turn exhibited
330 the lowest glycolytic rates (Figure 4). On the other hand, EIL supposedly having an intermediate
331 metabolism, exhibited also intermediate amounts of these compounds. The remarkably higher level
332 of anserine and carnosine in chicken breast meat is ascribable to its in vivo metabolic behavior that
333 makes the muscle more needy of endogenous buffers able to contrast the protons produced through
334 anaerobic glycolysis, resulting in a buildup of histidine compounds in the muscle (Puolanne and
335 Kivikari, 2000). According to this hypothesis, both thigh and drumstick muscles exhibited reduced
336 concentrations of histidine dipeptides because they do not necessitate to contrast large amount of

acidic end-products in vivo. These results seem to corroborate the strong relationship existing between the amounts of histidine dipeptides and the energy metabolism of muscle, as already suggested by previous studies conducted on porcine muscles (Cornet and Bousset, 1999; Mora et al., 2008). In this regard, Pearson correlation coefficients showed that both anserine and carnosine were highly positively correlated ($P<0.001$) with overall buffering capacity and glycolytic potential of chicken muscles (Table 1; see supplementary material S2 for Pearson correlation matrixes calculated for each muscle). Considering these aspects, it might be reasonable to assume that the content of histidine dipeptides might be one of the key factors regulating muscle post-mortem metabolism. Indeed, in virtue of its glycolytic potential (Figure 4) as well as the high contraction speed of its fast-twitch, glycolytic fibers, PM should have exhibited a faster and greater acidification within the first 120 min post-mortem (i.e. when muscle pH drops from 6.60 to 6.30), suggesting that the remarkable concentration of histidine dipeptides might have buffered a potentially stronger acidification in the first hour post-mortem. This hypothesis is further supported by anserine and carnosine's pK_a values that, being respectively 6.38 and 7.04, guarantee the maximal buffering capacity at pH ranges included from 6.4 to 7.0 (Boldyrev and Severin, 1990; Pösö and Puolanne, 2005) This scenario would confirm that histidine compounds exert their buffering activity not only in vivo, but also during post-mortem period, at least in the first hour after the death of the birds where muscle's pH is still close to its physiological value. Furthermore, it should be emphasized that, considering its glycogen content at 15 min, PM should have also exhibited lower pH_u values in absolute terms (<5.7-5.8). This trend further supports the hypothesis that histidine dipeptides might have limited not only the rate, but also the extent of early post-mortem acidification of PM muscle by buffering the acidic end-products of anaerobic glycolysis. Within this context, it is reasonable to speculate that the muscular concentration of histidine-containing compounds, having great outcomes on muscle buffering ability, might provide resistance to post-mortem pH decline, thus exerting an effect on muscle metabolism during pre-rigor phase and the quality of the forthcoming meat.

CONCLUSION

This study establishes the solid relationship existing between the content of anserine and carnosine and muscle post-mortem metabolism, indicating that the selection of PM, EIL and GI chicken muscles based on their histidine dipeptides thoroughly reflects their predominant energy-supplying metabolism. Being remarkably responsible for the buffering capacity of skeletal muscles, histidine dipeptides provide a resistance to post-mortem pH decline, thus explaining the slower and reduced extent of muscular acidification of PM muscle that, being markedly glycolytic, should have exhibited a lower pH_u in absolute terms. Thus, it could be hypothesized that the concentration of anserine and carnosine might also account for differences in pH_u values existing both within and between different mammalian and poultry muscles characterized by the similar energy metabolism.

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567

568 **TABLE**

569 **Table 1.** Pearson correlation coefficients between the overall concentration of histidine dipeptides,
570 buffering capacity and glycolytic potential assessed in chicken *Pectoralis major* (PM), *extensor*
571 *iliotibialis lateralis* (EIL) and *gastrocnemius internus* (GI) muscles (n=24). ***= P < 0.001

	Buffering capacity*	Glycolytic Potential
Anserine	+ 0.86***	+ 0.91***
Carnosine	+ 0.79***	+ 0.87***

572 * overall buffering capacity of PM, EIL and GI muscles calculated as the average of buffering capacity values detected in the pH
573 range 6.0-7.0.
574

FIGURE CAPTIONS

Figure 1. Muscles selected for the experiment and relative anatomic location.

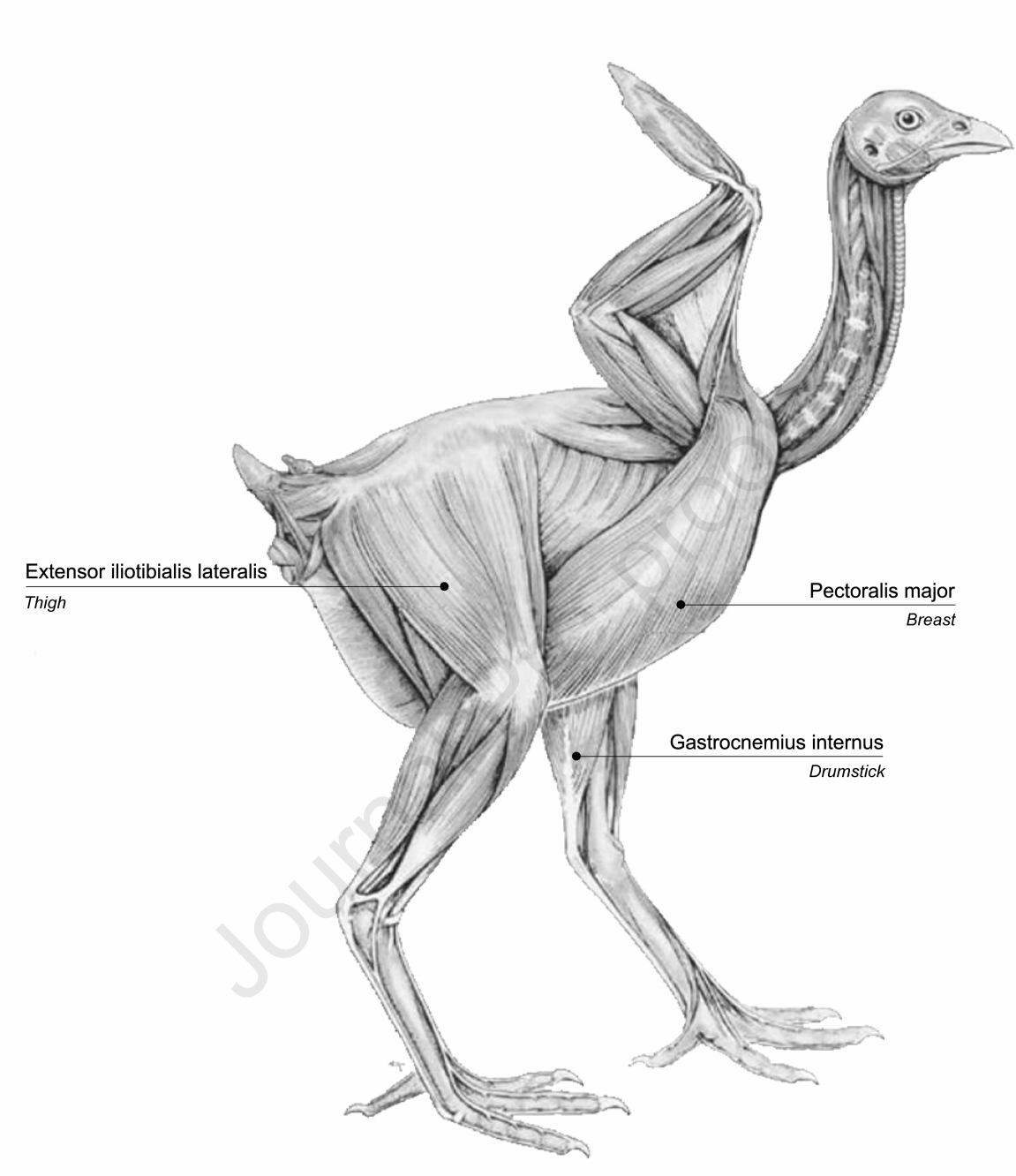
Figure 2. Average pH values of chicken *Pectoralis major* (PM), *extensor iliotibialis lateralis* (EIL) and *gastrocnemius internus* (GI) muscles at 15, 60, 120 and 1,440 min post-mortem (n=8/group). a-d: means lacking a common letter significantly differ among the time points within the same muscle ($P<0.05$). x-z= means lacking a common letter significantly differ among the muscles within the same time point ($P<0.05$). Error bars indicate standard error of means.

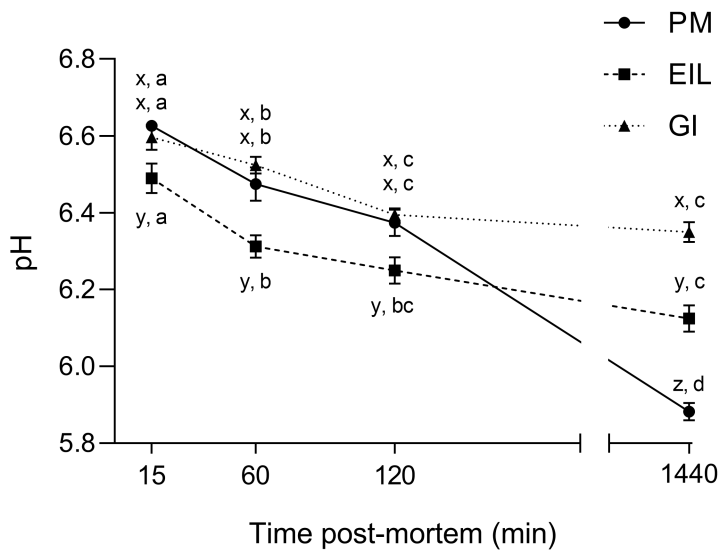
Figure 3. Average lactate (a, µmol/g), glycogen (b, µmol/g), glucose (c, µmol/g) and glucose-6-phosphate (d, µmol/g) of chicken *Pectoralis major* (PM), *extensor iliotibialis lateralis* (EIL) and *gastrocnemius internus* (GI) muscles (n=8/group) at 15, 60, 120 and 1,440 min post-mortem. a-c: means lacking a common letter significantly differ among the time points within the same muscle ($P<0.05$). x-z= means lacking a common letter significantly differ among the muscles within the same time point ($P<0.05$). Error bars indicate standard error of means.

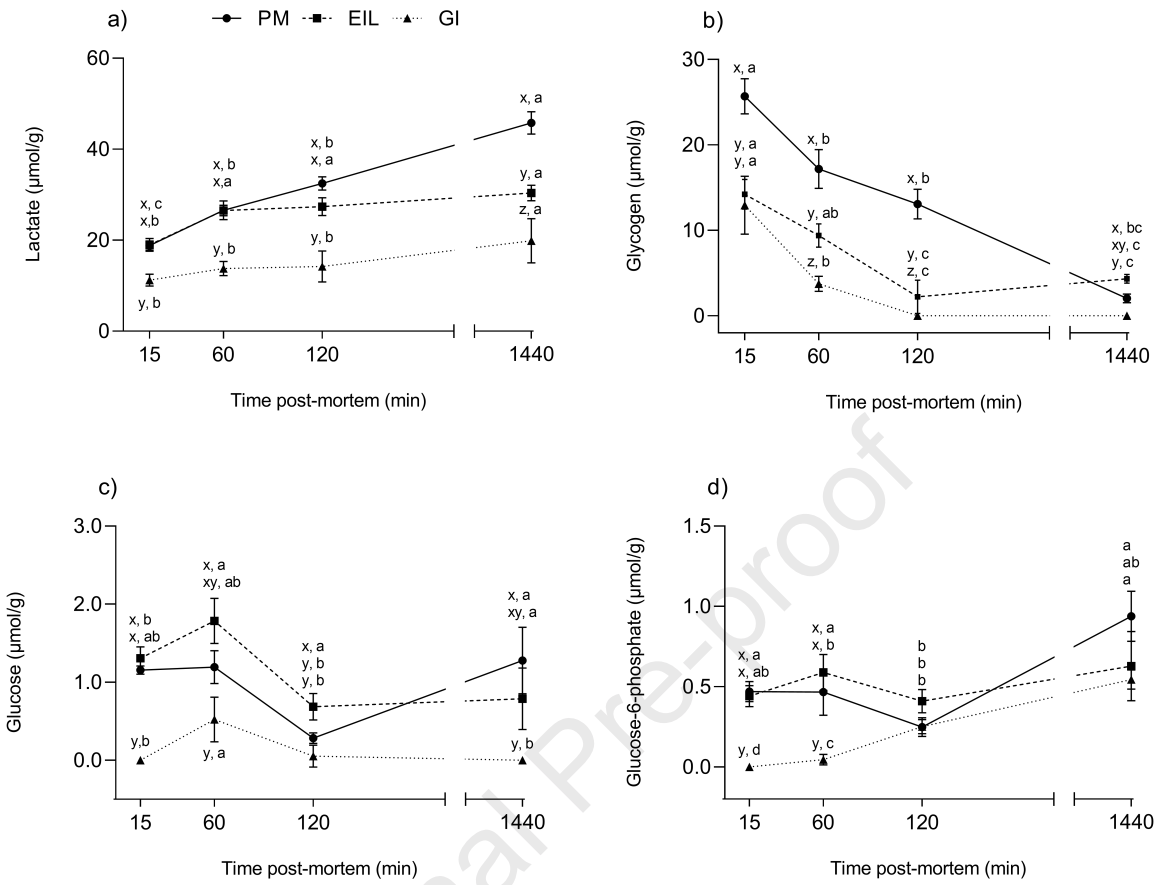
Figure 4. Average glycolytic potential (µmol lactate/g muscle) of chicken *Pectoralis major* (PM), *extensor iliotibialis lateralis* (EIL) and *gastrocnemius internus* (GI) muscles (n=8/group). a-c: means lacking a common letter significantly differ ($P<0.05$). Error bars indicate standard error of means.

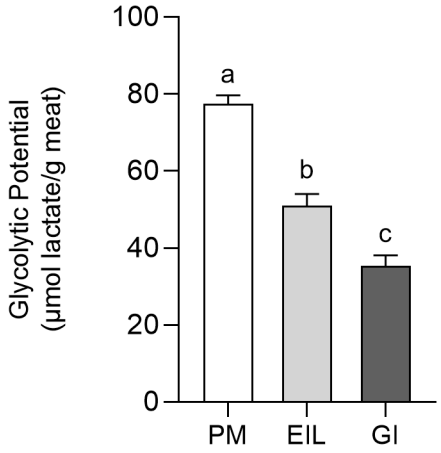
Figure 5. Buffering capacity (µmol $H+\cdot pH^{-1}\cdot g^{-1}$) (pH range 6.0-7.0) in chicken *Pectoralis major* (PM), *extensor iliotibialis lateralis* (EIL) and *gastrocnemius internus* (GI) muscles (n=8/group). a-c: means lacking a common letter significantly differ among the same pH range ($P<0.05$). Error bars indicate standard error of means.

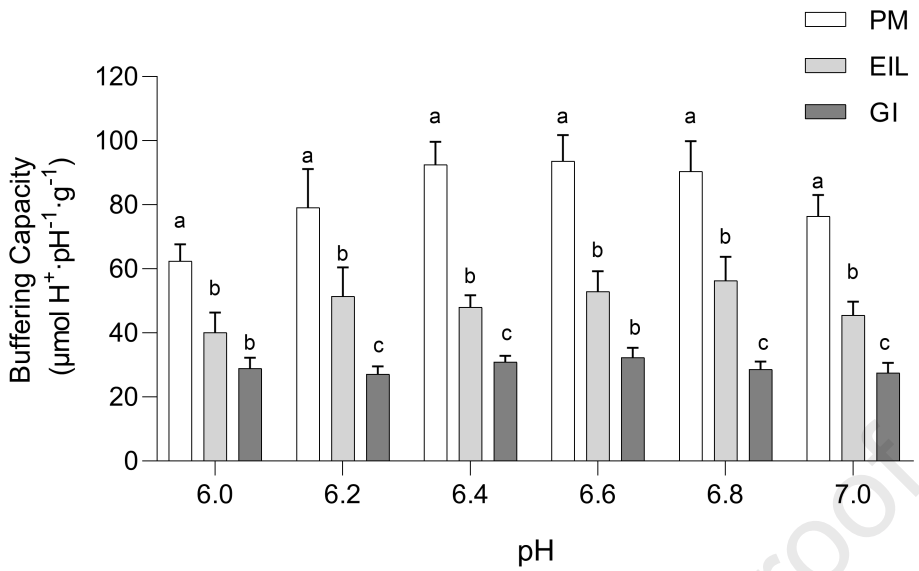
Figure 6. Average values of anserine and carnosine concentrations (mg/100g meat) in chicken *Pectoralis major* (PM), *extensor iliotibialis lateralis* (EIL) and *gastrocnemius internus* (GI) muscles (n=8/group). a-c: means lacking a common letter significantly differ among muscles ($P<0.05$). Error bars indicate standard error of means.

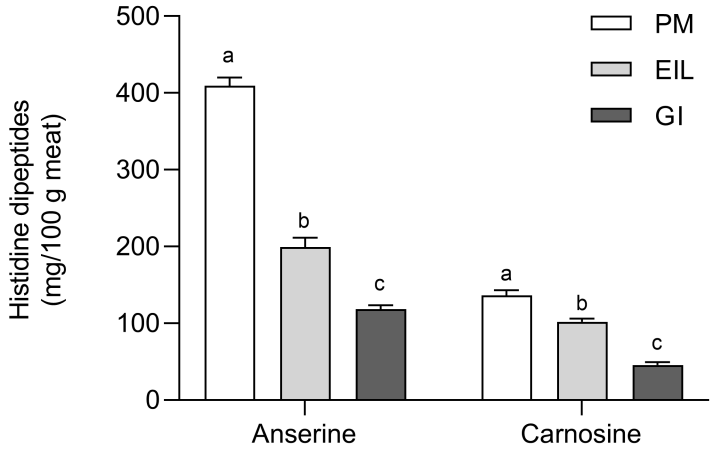












DEPARTMENT OF AGRICULTURAL AND FOOD SCIENCES

Editorial Office of Poultry Science Journal

Cesena, November 10th, 2020

Dear Editorial Office,

We have no conflict of interest to declare.

Sincerely yours,

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