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5
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Abstract

This study investigated the protein digestibility of chicken breast and thigh in an *in vitro* digestion model to determine the better protein sources for the elderly in terms of bioavailability. For this purpose, the biochemical traits of raw muscles and the structural properties of myofibrillar proteins were monitored. The thigh had higher pH, 10% trichloroacetic acid-soluble α-amino groups, and protein carbonyl content than the breast ($P<0.05$). In the proximate composition, the thigh had higher crude fat and lower crude protein content than the breast ($P<0.05$). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of myofibrillar proteins showed noticeable differences in the band intensities of tropomyosin α-chain and myosin light chain-3 between the thigh and breast. The intrinsic tryptophan fluorescence intensity of myosin was lower in the thigh than in the breast ($P<0.05$). Moreover, circular dichroism spectroscopy of myosin revealed that the thigh had higher α-helical and lower β-sheet structures than the breast ($P<0.05$). The cooked muscles were then chopped and digested in the elderly digestion model. The thigh had more α-amino groups than the breast after both gastric and gastrointestinal digestion ($P<0.05$). SDS-PAGE analysis of the gastric digesta showed that more bands remained in the digesta of the breast than that of the thigh. The content of proteins less than 3 kDa in the gastrointestinal digesta was also higher in the thigh than in the breast ($P<0.05$). These results reveal that chicken thigh with higher *in vitro* protein digestibility is a more appropriate protein source for the elderly than chicken breast.

Keywords: chicken breast, chicken thigh, protein digestibility, elderly digestion, dietary protein source

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1. Introduction

The trend of a rapidly aging society has attracted significant attention. By 2050, nearly two billion will be over 60 years old (United Nations, 2019). Therefore, strategies to deal with aging-related issues to support a good quality of life have become crucial. Food intake and dietary patterns are pivotal factors for healthy aging. Nutrition plays an important role in controlling age-related physiological changes and chronic diseases; inadequate nutritional consumption negatively affects health and impairs important functional abilities (Calligaris et al., 2022). Protein intake, which has a direct effect on muscle anabolism, is particularly important in the elderly population to prevent sarcopenia, which leads to frailty and disability due to the considerable decline in muscle mass (Lee et al., 2021a). However, all physical and physiological processes are greatly affected by aging, and in the elderly, unlike with young adults, protein digestion can be inhibited due to poor oral health and the deterioration of their gastrointestinal function (Hernández-Olivas et al., 2020). In this context, the selection of a dietary protein source tailored to the specific health status of the elderly can prevent malnutrition by maintaining a nutritionally balanced diet and reducing several health problems.

Poultry meat, especially chickens with high protein content, has attracted attention due to consumer concerns about cardiovascular diseases caused by the overconsumption of red meat (Shin & Choi, 2022; Zou et al., 2018a). The low caloric property of chicken meat due to its lower fat content compared to other types of meat also appeals to consumers for a healthier diet (Barido et al., 2021; Kralik et al., 2018; Park et al., 2022). Moreover, a study by Hernández-Olivas et al. (2022) suggested that chicken meat is an ideal choice for the elderly among four different types of meat (beef entrecote, pork loin, turkey breast, and chicken breast) in terms of protein digestibility. Among the different chicken cuts, breasts and thighs have a significant preference and popularity among consumers (Haley, 2001). These two cuts consist of a distinct composition of muscle fibers that represent characteristics of slow- and fast-twitch muscle

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fibers, depending on their muscle contraction speed and respiration activity (Xiong, 1994). Myosin, a representative protein with different isoforms depending on the muscle fiber types, participates in muscle contraction with other myofibrillar proteins, such as tropomyosin and troponin, and is generally considered as the molecular marker of fiber types (Choi & Kim, 2009). Myosin is composed of two myosin heavy chains (MHC) and four myosin light chains (MLC) that regulate myosin motor activity. Among the several types of myosin isoforms, four of them (including I, IIA, IIX, and IIB) are the predominant MHC isoforms in skeletal muscle. MHC I and the other three isoforms are expressed in slow-twitch (oxidative or type I) and fast-twitch (glycolytic or type II) muscle fibers, respectively (Pette & Staron, 2000) and the avian skeletal muscle is primarily composed of these two types of muscle fibers.

Although the content of each muscle fiber varies depending on experimental method, breed, age, and study, a higher slow-twitch muscle fiber content in leg muscles, including the thigh, has been consistently reported. Verdiglione and Cassandro (2013) suggested that the breast muscle of broilers comprises 100% fast-twitch fibers, whereas Du et al. (2017) observed 76%–79% slow-twitch fibers in broiler legs. Jaturasitha et al. (2008), who monitored the muscle composition of Thai native chickens, reported that the fiber count of type I was 1.1% and 18.2% of the total in the breast and thigh muscles, respectively. Moreover, type IIA (3.9% and 26.0% of the total in the breast and thigh, respectively) and IIB (95.0% and 55.8% of the total in the breast and thigh, respectively) also showed considerably different values. Apart from the differences in the fiber types, in the case of chicken thigh, the presence of the various types of muscles might also contribute to the distribution of various types of proteins, unlike chicken breast.

As a result of this, we assumed that the thigh and breast have different digestive characteristics due to their metabolic, contractile, and enzymatic properties. Therefore, it is useful to monitor their biochemical properties to understand the differences in the protein

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bioavailability of the two muscles. Although several studies have compared the *in vitro* protein digestibility between meat sources, research on the digestive behavior of proteins in different muscle fiber types is limited. Zou et al. (2018b) reported that the different muscle fiber types can be attributed to the different compositions of muscle proteins and their digestive susceptibility. Moreover, since the muscle fibers have different resistance to thermal denaturation (Vaskoska et al., 2021), we hypothesized that chicken breast and thigh may have different digestive behavior especially after cooking. Therefore, this study aims to investigate the structural and functional properties of chicken breast and thigh muscle. In addition, we evaluate the *in vitro* protein digestibility of the two muscles in an elderly digestion model to determine the muscle with ideal protein source.

2. Materials and methods

2.1. Sample preparation

Five commercially processed broiler carcasses were purchased from a local market (Daejeon, Korea). The breast fillets and thighs (mainly composed of *iliofibularis* and *iliotibialis*) were removed from the carcasses along with excessive fat and connective tissues, followed by grinding (CH580, Kenwood Ltd., Havant, UK) for the experimental analysis. Each ground muscle was vacuum-packed and stored at −70°C after sampling until analysis.

2.2. pH

The pH of the muscles was monitored immediately after sample collection. An aliquot (9 mL) of distilled water was added to 1 g of muscle, followed by homogenization (T25 basic, IKA-Werke GmbH & Co. KG, Staufen, Germany) for 30 s. The homogenate was centrifuged at 2,000 × g (1580R, LABOGENE, Lynge, Denmark) and the supernatant was filtered through

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filter paper (No. 4, Whatman, Maidstone, UK). The pH of the filtrate was measured using a pH meter (SevenEasy, Mettler-Toledo Intl. Inc., Schwerzenbach, Switzerland).

2.3. Proximate composition

The proximate composition including moisture, crude protein, crude fat, and crude ash content of the chicken muscles was monitored according to AOAC methods 950.46, 928.08, 960.39, and 923.03, respectively.

2.4. α-Amino group content

A 10% trichloroacetic acid (TCA)-soluble fraction was obtained according to the method described by Lee et al. (2020a). The α-amino group content in the TCA-soluble fraction and digesta was monitored through reaction with o-phthaldialdehyde (OPA) reagent (Church et al., 1983). The absorbance of the mixture was measured at 340 nm using a plate reader (Varioskan LUX, Thermo Fisher Scientific, Waltham, MA, USA). A standard curve was produced with glycine, and the crude protein content was measured using the Kjeldahl method (AOAC method 928.08).

2.5. Protein carbonyl content

Protein carbonyl content was monitored to evaluate protein oxidation, following the method described by Estévez (2011). Chicken muscle (1 g) was homogenized with 10 mL of 0.6 M NaCl in 20 mM sodium phosphate (pH 6.5) at 13,000 rpm for 30 sec (T25 basic). The mixture was used as the sample to monitor protein carbonyl content. The samples were reacted with dinitrophenylhydrazine and the absorbance of the mixtures were read at 370 nm. Protein content was measured using a Bio-Rad protein assay (#5000006, Bio-Rad Laboratories, Inc., Richmond, CA, USA). Bovine serum albumin was used to create a standard curve and the

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131 absorbance was read at 595 nm. The protein carbonyl content was calculated using a molar
132 absorptivity of 22,000 M^{-1} cm^{-1} and expressed in nmol carbonyl/mg protein.
133
134 **2.6. Myofibrillar protein extraction**

135 Myofibrillar proteins were prepared according to the method of Liu and Xiong (1996). The
136 chicken muscle (3 g) was homogenized with 25 mL of ice-cold buffer containing 0.1 M KCl,
137 2 mM $MgCl_2$, and 1 mM ethylene glycol tetraacetic acid (T25 basic) at 13,000 rpm for 30 sec.
138 The homogenate was centrifuged at 3,000 × g for 10 min (1580R) to remove the supernatant
139 and this procedure was repeated three times. Then, the pellet was homogenized with 20 mL of
140 0.1 M NaCl and centrifuged to remove the supernatant three times. The final pellet was
141 suspended in a 0.1 M potassium phosphate buffer (pH 7.4). The protein content was measured
142 using a Bio-Rad protein assay.
143
144 **2.7. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)**

145 The myofibrillar protein extracts were diluted to a concentration of 2 mg/mL for SDS-PAGE
146 analysis. The myofibrillar protein samples were reacted with a sample buffer (EBA-1051, Elpis
147 Biotech Inc., Daejeon, Korea) at a ratio of 1:1 (vol/vol) at 90°C for 10 min. Electrophoretic
148 separation was conducted using 12.5% polyacrylamide gel in an electrophoresis system (AE-
149 6531 mPAGE, ATTO Co., Ltd., Tokyo, Japan) and a protein ladder (3454A, Takara Bio Inc.,
150 Shiga, Japan) was loaded to figure out the molecular weights of protein bands. The protein
151 bands were stained using a solution containing Coomassie brilliant blue and de-stained using
152 a solution containing 10% acetic acid and 30% methanol. The SDS-PAGE gel was scanned
153 using Epson Perfection V850 Pro (V850 Pro, Epson, Long Beach, CA, USA).
154
155 **2.8. Myosin extraction**

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156 Myosin was extracted using the method previously reported by Reddish et al. (2005) with
157 some modification. The chicken muscle (1 g) was solubilized in buffer solution with high
158 content of salt (0.04 M sodium pyrophosphate, 1 mM $MgCl_2$, and 2 mM ethylene diamine
159 tetraacetic acid (EDTA), pH 9.5) and then precipitated by the buffer solution with low content
160 of salt (0.02 M KCl, 2 mM KH_2PO_4, and 1 mM EDTA, pH 6.8). The final pellets were
161 suspended in 0.6 M KCl in a 20 mM potassium phosphate buffer (pH 7.0), and the protein
162 content was measured using a Qubit Protein Assay Kit (A50669, Thermo Fisher Scientific,
163 Waltham, MA, USA).
164

165 **2.9. Intrinsic tryptophan fluorescence intensity**

166 The intrinsic tryptophan fluorescence intensity of myosin extract (protein concentration of
167 0.5 mg/mL) was observed using the method reported by Lee et al. (2021b). Scanning of the
168 myosin extract was conducted between 300 and 400 nm at an excitation wavelength of 280 nm
169 (Varioskan LUX), with a scanning speed of 1,000 nm/min, and excitation and emission slit
170 widths of 5 nm.
171

172 **2.10. Circular dichroism spectroscopy**

173 The secondary structure of the myosin extract was measured using circular dichroism
174 spectroscopy (Chirascan VX, Applied Photophysics Ltd., Leatherhead, UK). The myosin
175 extract was diluted to a protein concentration of 0.5 mg/mL. A single spectrum was obtained
176 with two replicates in a scan rate of 100 nm/min, a response time of 0.25 s, and a bandwidth of
177 1.0 mm. The data were expressed in millidegrees, and the scanning range was set between 200
178 and 260 nm. To estimate the content of secondary structural components, including α-helices,
179 β-sheets, β-turns, and random coils in the spectrum, CDNN software (version 4.0, Gerald Böhm,
180 Bioinformatics, Germany, CD Spectra Deconvolution Ver. 2.1) was used.

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182 **2.11. *In vitro* digestion**

183 For *in vitro* digestion, the ground chicken muscles were vacuum-packed and cooked at 80°C 184 to reach a core temperature of 75°C, followed by cooling to 25°C. Cooked samples were 185 chopped to simulate mastication.

186 The elderly *in vitro* digestion model herein was designed based on previous studies by 187 Hernández-Olivas et al. (2020) and Minekus et al. (2014). All the digestive enzymes that were 188 used herein were purchased from Sigma-Aldrich (St. Louis, MO, USA). The simulated salivary 189 fluid (pH 7.0), gastric fluid (pH 6.0), and duodenal fluid (pH 7.0) contained 75 U/mL α-amylase 190 from *Aspergillus oryzae* (EC 3.2.1.1), 1,500 U/mL pepsin from *Porcine mucosa* (EC 3.4.23.1), 191 50 U/mL trypsin (EC 3.4.21.4) and 12.5 U/mL chymotrypsin (EC 3.4.21.1) from bovine 192 pancreas, 1,000 U/mL pancreatic lipase from porcine pancreas (EC 3.1.1.3), and 5 mM porcine 193 bile extract (EC 232-369-0). Digestive fluid was mixed with the digesta from the previous 194 compartment at 50:50 (vol/vol) during digestion. Each digestion was conducted for 120 min at 195 37°C and a rotational speed of 100 rpm, except for oral digestion, which was conducted for 2 196 min. All digesta samples were stored at −70°C until analysis, immediately after digestion. 197 Control samples were prepared for digestion under the same conditions through addition of 198 distilled water instead of meat samples to exclude protein content from the digestive enzymes.

199 Herein, the size fractionation of the digesta was conducted to determine protein digestibility 200 after *in vitro* digestion. After sequential filtration using a centrifugal filter with molecular 201 weight cut-offs of 10 and 3 kDa (Amicon Ultra-15, Millipore, Billerica, MA, USA) according 202 to the manufacturer's protocol, the protein content of the filtrate and whole digesta was 203 measured using the Kjeldahl method to represent the amount of protein digested under 3 kDa. 204 Protein digestibility was calculated using the following equation:

205 Digestibility (%) = 100 × (protein content in the filtrate − protein content of the control

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206 filtrate) / protein content in the whole digesta.

207

208 **2.12. Statistical Analysis**

209 This study used three iterations in three batches with analysis using a mixed model, and the
210 batches (carcasses) were described as random effects. The least-squares mean and standard
211 error of the least-squares mean was used to express the results. The significance of the main
212 effects was evaluated using Tukey's multiple comparison test (P<0.05). The results were
213 statistically analyzed using SAS software (version 9.3, SAS Institute, Inc., Cary, NC).

214

215 **3. Results and discussion**

216 **3.1. Muscle characterization**

217 Table 1 shows the results of the muscle characterization in this study. The pH of chicken
218 breast and thigh used in this study was 5.81 and 6.49, respectively, with significantly higher
219 pH in the thigh (P<0.05). The breast and thigh showed different proximate compositions, with
220 higher crude protein and crude ash contents in the breast (P<0.05), and conversely, higher crude
221 fat content in the thigh (P<0.05). Moreover, the thigh had higher protein carbonyl content than
222 the breast (P<0.05).

223 Muscle fiber type differentiates the contractile characteristics and metabolic patterns
224 (glycolytic and oxidative) that influence muscle-to-meat conversion and overall meat quality.
225 As the slow- and fast-twitch muscle fibers have oxidative and glycolytic metabolism before
226 slaughter, respectively, muscles predominantly composed of fast-twitch fibers possess more
227 glycogen than those composed of slow-twitch fibers (Karlsson et al., 1999). The lower
228 glycogen content in type I fibers can inhibit pH decline in muscles that are rich in type I fibers
229 after slaughter (Vaskoska et al., 2021), which explains the higher pH of the thigh. Moreover,
230 compared to fast-twitch fibers, slow-twitch fibers have a greater ability to use cellular lipids as

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231 fuel for ATP production due to their high mitochondria content and respiratory enzymes,
232 resulting in high triglyceride content in muscle fibers (Karlsson et al., 1999). Therefore, the
233 higher stored triglyceride content in the thigh resulted in higher crude fat and lower crude
234 protein content than in the breast. This oxidative metabolic property of slow-twitch fibers also
235 requires a greater ability to deliver oxygen to the muscle; as such, the chicken thigh has a higher
236 heme protein content (including hemoglobin and myoglobin) than the breast (Gong et al., 2010).
237 As heme proteins and transition metal ions are important factors that affect the oxidative
238 susceptibility of meat proteins (Jongberg et al., 2014), the metal ion-induced acceleration of
239 protein oxidation results in higher protein carbonyl content in the thigh.

240 Additionally, the α-amino group content in the 10% TCA-soluble fraction that contained
241 small amino acids and peptides with 3-4 residues was higher in the thigh ($P<0.05$), indicating
242 greater postmortem protein degradation. In general, fast-twitch fibers are considered to have
243 faster postmortem proteolysis due to having a higher calpain-to-calpastatin ratio than slow-
244 twitch fibers, as calpastatin activity is positively correlated with slow MHC isoforms
245 (Christensen et al., 2004). However, calpain is highly sensitive to pH and exhibits optimum
246 activity at neutral pH (Bhat et al., 2018). The rapid pH decline and lower ultimate pH of the
247 breast may have induced lower calpain activity and an increase in protein denaturation,
248 resulting in a greater breakdown of proteins in the thigh. However, Christensen et al. (2004)
249 reported that the postmortem degradation of proteins depends more on the variations between
250 muscles rather than the fiber type itself, and that the difference in the α-amino group content
251 cannot be entirely explained by muscle fiber composition.

252 Overall, the two muscles exhibit different biochemical traits, which may be attributed to
253 differences in their metabolic processes.

254

255 **3.2. Myofibrillar protein structure of chicken breast and thigh muscles**

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The previous section confirmed that chicken breast and thigh muscles have different muscle properties. To understand the difference in the digestive susceptibility of the two muscles, their structural characteristics and biochemical traits should be determined, because protein structure is an important factor that determines the accessibility of digestive enzymes to their cleavage sites in meat proteins (Lee et al., 2021a). Therefore, we will discuss the structural differences in the myofibrillar proteins, which are the major proteins in the muscle, between the two muscles.

3.2.1. SDS-PAGE of myofibrillar proteins in chicken breast and thigh muscles

The SDS-PAGE electrophoretogram of myofibrillar proteins showed different protein bands between the breast and thigh (Figure 1A). The most noticeable difference was observed in the tropomyosin α- and β-chains. Although the tropomyosin β-chain appeared in both lanes, there was no tropomyosin α-chain in the thigh myofibrillar protein lane. In myofibrillar proteins, components such as troponin or actin exist as a single isoform, whereas others such as myosin and tropomyosin exist in several isoforms, which may differ depending on the muscle fiber type (Schevzov et al., 2011). According to a study by Heeley et al. (1985), although the α- and β-subunits of tropomyosin are the predominant components in both slow- and fast-twitch muscle fibers at birth, differentiation into slow-twitch fibers is accompanied by a decline in α-tropomyosin content. Therefore, the absence of the tropomyosin α-chain band in the thigh can be explained by the higher slow-twitch muscle fiber content compared to the breast. Moreover, the myosin light chain-3 band was weaker in the breast than in the thigh (Figure 1A). Stuart et al. (2016) reported that although myosin light chain-1 is predominant in all fiber types, myosin light chain-3 is almost non-existent in fast-twitch muscle fibers. This result suggests that myosin light chain-3 is an essential phenotype of type I muscle fibers.

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3.2.2. Intrinsic tryptophan fluorescence intensity of myosin in chicken breast and thigh muscles

The hydrophobicity of proteins is known to largely contribute to their structural stability. The intrinsic fluorescence intensity of tryptophan, an aromatic amino acid, was monitored to determine the aromatic hydrophobicity of myosin in chicken breast and thigh tissues (Figure 2). In the wavelength range of 300 to 400 nm, the breast muscle had a higher intrinsic tryptophan fluorescence intensity than the thigh muscle (Figure 2A). When comparing the fluorescence intensity at 328 nm, which showed the highest intensity in both muscles (Figure 2B), the breast had a significantly higher fluorescence intensity (P<0.05). This result indicates that the thigh has a higher aromatic surface hydrophobicity than the breast, which is consistent with previous studies. Boyer et al. (1996) reported that both aromatic and aliphatic hydrophobicity was higher in slow-twitch myosin than in fast-twitch myosin; the study showed that the aromatic surface hydrophobicity of slow-twitch myosin was 1.5-fold higher than that of fast-twitch myosin. Glorieux et al. (2017) also reported higher aromatic surface hydrophobicity in the thigh due to different myosin isoforms in the two muscles. Therefore, it appears that slow-twitch myosin has more hydrophobic residues on its surface than fast-twitch myosin. As the hydrophobic residues exposed on the surface form aggregates with hydrophobic interactions by thermal treatment (Mitra et al., 2017), the higher surface hydrophobicity of the chicken thigh can negatively influence the digestive susceptibility of proteins. This is further discussed in the succeeding sections.

3.2.3. Secondary structure of myosin in chicken breast and thigh muscles

The relative percentages of the secondary structural components of myosin in the chicken breast and thigh are presented in Table 2. Myosin in the thigh had higher α-helix content and lower β-sheet content than that in the breast (P<0.05). There was no significant difference in

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306 the β-turn and random coil composition between the two muscles (*P*>0.05). Although few
307 studies have compared and discussed the secondary structure of myosin between muscle fiber
308 types, muscles primarily consisting of slow-twitch fibers were reported to have a higher α-
309 helical structure than muscles with a relatively high fast-twitch fiber content (Bozkurt et al.,
310 2010; Katemala et al., 2021). This difference in myosin structure may have been caused by the
311 different isoforms. Though both α-helices and β-sheets are ordered structures, β-sheets
312 generally represent a more compact and aggregated structure with intramolecular hydrogen
313 bonding (Tan et al., 2021). Moreover, as α-helices are often positively correlated with protein
314 digestibility (Bai et al., 2016; Han et al., 2019), this difference in the secondary structure of
315 myosin between the two muscles can also contribute to digestive characteristics.
316

317 **3.3. *In vitro* protein digestibility of chicken breast and thigh muscles**

318 The *in vitro* digestion in the elderly digestion model was divided into two compartments for
319 gastric and gastrointestinal (adding simulated duodenal fluids following gastric digestion)
320 digestion, as meat proteins are reported to be digested in different stages of digestion (Lee et
321 al., 2021a). We employed three experimental parameters in the digesta to determine differences
322 in the digestive characteristics of the two muscles. The α-amino group content was used to
323 measure the release of small amino acids and peptides during *in vitro* digestion (Figure 3A). In
324 the SDS-PAGE electrophoretogram of the gastric and gastrointestinal digesta, the protein that
325 can be preferentially digested is estimated by the disappearance of protein bands (Figure 1B).
326 As protein bioavailability is much more important in the elderly for protein anabolism and
327 prevention of sarcopenia (Lee et al., 2021c), we also monitored the protein content digested
328 below 3 kDa (Figure 3B). A previous study reported that proteins with a molecular weight less
329 than 3 kDa can be absorbed in the small intestine (Vlahou et al., 2018); therefore, they can be
330 used to evaluate the amount of protein absorbed after digestion.

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331 The thigh had higher α-amino group content than the breast after gastric and gastrointestinal
332 digestion (Figure 3A, $P<0.05$), and the difference between the two muscles increased after
333 gastrointestinal digestion. The protein content digested below 3 kDa after gastrointestinal
334 digestion was also higher in the thigh than in the breast (Figure 3B, $P<0.05$). Therefore, in the
335 elderly digestion model, the thigh had higher *in vitro* protein digestibility than the breast.

336 The higher *in vitro* protein digestibility in the thigh can be explained by the difference in
337 thermal stability between the two muscles, as we cooked the chicken muscles at 80°C before
338 *in vitro* digestion because meat is generally cooked before consumption. Following this, we
339 discovered higher intrinsic tryptophan fluorescence intensity in the breast than in the thigh,
340 indicating higher aromatic surface hydrophobicity of the thigh. In general, exposed
341 hydrophobic residues form aggregates via hydrophobic interactions during thermal treatment
342 (Mitra et al., 2017), potentially resulting in a decrease in protease accessibility (Lee et al.,
343 2021a). However, according to the results of Boyer et al. (1996), although the surface
344 hydrophobicity of slow-twitch myosin before heating is higher, fast-twitch myosin shows
345 higher hydrophobicity after thermal treatment, indicating higher susceptibility of the
346 hydrophobic residues in fast-twitch myosin to the heat-induced protein unfolding. This
347 explanation is consistent with the results of Vaskoska et al. (2021), who compared the thermal
348 stability between muscles primarily comprised fast- and slow-twitch muscles; the study
349 reported that fast-twitch muscle fibers have lower thermal stability with an earlier onset of
350 thermal denaturation and a greater extent of aggregated strands after cooking compared to
351 slow-twitch fibers. Young et al. (1992) also reported a lower (by 10°C) heat-induced gelation
352 temperature of myofibrillar proteins from fast-twitch fibers. This difference in thermal stability
353 between the two fiber types may be related to their different amino acid compositions
354 influencing the primary structure; for instance, slow-twitch myosin containing various
355 substitutions in the light chain region with a shorter sequence of amino acids (Chikuni et al.,

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356 2004). Moreover, the lower ultimate pH of the breast may have also affected the lower thermal
357 stability, as Böcker et al. (2006) reported that more aggregated strands were observed in
358 muscles with lower pH. We also observed the secondary structure in Table 2, wherein the breast
359 had lower α-helix content and higher β-sheet content than the thigh. Thus, the breast may have
360 a more compact structure and a lower protease accessibility, even before cooking. As heat-
361 induced protein aggregation can hinder the action of digestive enzymes via protein aggregation
362 followed by the burial of their cleavage sites (Lee et al., 2021a), this may have resulted in lower
363 protein digestibility in the breast than in the thigh.

364 Therefore, aggregation-induced steric hindrance of myofibrillar proteins and digestive
365 enzymes may have been greater in the breast. In the SDS-PAGE electrophoretogram of the
366 digesta (Figure 1B), although there were no noticeable differences in the protein bands of the
367 gastrointestinal digesta, there were more bands in the gastric digesta of the breast than that in
368 the thigh. In the gastric digesta, the actin band appeared with a relatively higher intensity than
369 any other protein, indicating the digestive resistance of actin in the gastric phase, as we have
370 already observed in our previous studies (Lee et al., 2020b, 2021b). In particular, the breast
371 still had troponin and tropomyosin α- and β-chains though they disappeared in the thigh.
372 Troponin and tropomyosin are associated with actin filaments by stabilizing the filament and
373 controlling the access of actin-binding proteins (Schevzov et al., 2011). Therefore, the
374 degradation of these two proteins can improve the digestive accessibility of myofibrillar
375 filaments by reducing their structural integrity and steric hindrance (Lee et al., 2021a).
376 However, as we have already discussed previously, the breast, which may have a more compact
377 and aggregated structure than the thigh, appears to have lower digestive susceptibility to
378 regulatory proteins, resulting in a lower overall digestion of myofibrillar proteins. The higher
379 digestion of the thigh in the gastric phase may also have induced greater digestion in the
380 intestinal phase due to more disrupted and fragmented protein structure.

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381 Aging is accompanied by the deterioration in the masticatory capacity and secretion of
382 digestive juices that can impair protein digestion. The delay in digestion due to the elevated
383 intragastric pH and deteriorated secretion of digestive enzymes contributes to slow gastric
384 emptying in the elderly (Lee et al., 2021a). The impairment in protein digestibility reduces the
385 absorbable amount of proteins so that the health problems such as sarcopenia and malnutrition
386 occur. This is why the elders are advised to select the adequate amount and appropriate types
387 of dietary proteins so that they can meet their protein requirements. Overall, the differences in
388 the metabolic and contractile properties of the two muscles can be attributed to their different
389 characteristics and structural properties. The chicken thigh, which was reported to have higher
390 slow-twitch muscle fibers than the breast, showed faster and greater protein digestion in the
391 elderly digestion model, resulting in higher *in vitro* protein digestibility. Therefore, the higher
392 and faster digestion of chicken thigh than chicken breast can be a desirable property to improve
393 overall digestive processes in the elderly. This result suggests that chicken thighs can be a better
394 choice as a dietary protein source for the elderly due to their higher protein bioavailability.
395 Although we have focused on the difference in myofibrillar proteins and the resulting *in vitro*
396 protein digestibility of two muscles herein, different muscular compositions can also attribute
397 to the different distribution of sarcoplasmic and stromal proteins. Therefore, future study is
398 required to compare the overall distribution of meat proteins and resulting protein
399 bioavailability in the muscles with different fiber compositions.
400
401 **4. Conclusion**
402 We aimed to compare *in vitro* protein digestibility between chicken breast and thigh muscles
403 with different muscle properties in an elderly digestion model. As hypothesized, chicken
404 breasts and thighs exhibit different biochemical and structural traits. In proximate composition,
405 chicken thigh had higher crude fat and lower crude protein contents than chicken breast and

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406 protein carbonyl and 10% TCA-soluble α-amino group contents were higher in chicken thigh
407 than in chicken breast. After *in vitro* digestion in the elderly digestion model, both the contents
408 of the α-amino groups and the proteins digested under 3 kDa were higher in the thigh, indicating
409 higher *in vitro* protein digestibility in the thigh than in the breast. Considering the absorption
410 and availability of ingested proteins, the chicken thigh is therefore potentially a better choice
411 than the chicken breast as a dietary protein source for the elderly due to its higher *in vitro*
412 protein digestibility.
413

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References

Bai M, Qin G, Sun Z, Long G. 2016. Relationship between molecular structure characteristics of feed proteins and protein in vitro digestibility and solubility. Asian-Australas J Anim Sci 29(8): 1159.

Barido FH, Jang A, Park JI, Kim YJ, Lee SK. 2021. The effect of pre-treated black garlic extracts on the antioxidative status and quality characteristics of Korean ginseng chicken soup (samgyetang). Food Sci Anim Resour 41(6): 1036-1048.

Bhat ZF, Morton JD, Mason SL, Bekhit AEDA. 2018. Role of calpain system in meat tenderness: A review. Food Sci Hum Wellness 7(3): 196-204.

Böcker U, Ofstad R, Bertram HC, Egelandsdal B, Kohler A. 2006. Salt-induced changes in pork myofibrillar tissue investigated by FT-IR microspectroscopy and light microscopy. J Agric Food Chem 54(18): 6733-6740.

Boyer C, Joandel S, Ouali A, Culioli J. 1996. Determination of surface hydrophobicity of fast and slow myosins from rabbit skeletal muscles: Implication in heat-induced gelation. J Sci Food Agric 72(3): 367-375.

Bozkurt O, Severcan M, Severcan F. 2010. Diabetes induces compositional, structural and functional alterations on rat skeletal soleus muscle revealed by FTIR spectroscopy: a comparative study with EDL muscle. Analyst 135(12): 3110-3119.

Calligaris S, Moretton M, Melchior S, Mosca AC, Pellegrini N, Anese M. 2022. Designing food for the elderly: the critical impact of food structure. Food Funct 13: 6467-6483.

Chikuni K, Muroya S, Nakajima I. 2004. Myosin heavy chain isoforms expressed in bovine skeletal muscles. Meat Sci 67 (1): 87-94.

Choi YM, Kim BC. 2009. Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. Livest Sci 122(2-3): 105-118.

Christensen M, Henckel P, Purslow P. 2004. Effect of muscle type on the rate of post-mortem

ACCEPTED

439 proteolysis in pigs. Meat Sci 66(3): 595-601.
440 Church FC, Swaisgood HE, Porter DH, Catignani GL. 1983. Spectrophotometric assay using
441 o-phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins. J
442 Dairy Sci 66(6): 1219-1227.
443 Du YF, Ding QL, Li YM, Fang WR. 2017. Identification of differentially expressed genes and
444 pathways for myofiber characteristics in soleus muscles between chicken breeds differing in
445 meat quality. Anim Biotechnol 28(2): 83-93.
446 Estévez M. 2011. Protein carbonyls in meat systems: A review. Meat Sci 89(3): 259-279.
447 Glorieux S, Steen L, Paelinck H, Foubert I, Fraeye I. 2017. Isothermal gelation behavior of
448 myofibrillar proteins from white and red chicken meat at different temperatures. Poult
449 Sci 96(10): 3785-3795.
450 Gong Y, Parker RS, Richards MP. 2010. Factors affecting lipid oxidation in breast and thigh
451 muscle from chicken, turkey and duck. J Food Biochem 34(4): 869-885.
452 Haley MM. 2001. Changing consumer demand for meat: the US example, 1970-2000. In
453 Changing Structure of Global Food Consumption and Trade. Economic Research Service,
454 USA. pp 42-48.
455 Han Z, Zhang J, Li C, Zhao D, Yue X, Shao JH. 2019. Effect of secondary heat-induced
456 aggregation on pork meat batter protein conformation, hydration characteristics, textural
457 quality and in vitro digestibility. Food Funct 10(11): 7407-7415.
458 Heeley DH, Dhoot GK, Perry SV. 1985. Factors determining the subunit composition of
459 tropomyosin in mammalian skeletal muscle. Biochem J 226(2): 461-468.
460 Hernández-Olivas E, Muñoz-Pina S, Sánchez-García J, Andrés A, Heredia A. 2020.
461 Understanding the role of food matrix on the digestibility of dairy products under elderly
462 gastrointestinal conditions. Food Res Int 137: 109454.
463 Hernández-Olivas E, Muñoz-Pina S, García-Hernández J, Andrés A, Heredia A. 2022. Impact
ACCEPTED

464 of common gastrointestinal disorders in elderly on in vitro meat protein digestibility and
465 related properties. Food Biosci 46: 101560.
466 Jaturasitha S, Kayan A, Wicke M. 2008. Carcass and meat characteristics of male chickens
467 between Thai indigenous compared with improved layer breeds and their crossbred. Arch
468 Anim Breed 51(3): 283-294.
469 Jongberg S, Wen J, Tørngren MA, Lund MN. 2014. Effect of high-oxygen atmosphere
470 packaging on oxidative stability and sensory quality of two chicken muscles during chill
471 storage. Food Packag Shelf Life 1(1): 38-48.
472 Karlsson AH, Klont RE, Fernandez X. 1999. Skeletal muscle fibres as factors for pork
473 quality. Livest Prod Sci 60(2-3): 255-269.
474 Katemala S, Molee A, Thumanu K, Yongsawatdigul J. 2021. Meat quality and Raman
475 spectroscopic characterization of Korat hybrid chicken obtained from various rearing
476 periods. Poult Sci 100(2): 1248-1261.
477 Kralik G, Kralik Z, Grčević M, Hanžek D. 2018. Quality of chicken meat. Anim Husb Nutr 63.
478 Lee S, Choi YS, Jo K, Yong HI, Jeong HG, Jung S. 2021a. Improvement of meat protein
479 digestibility in infants and the elderly. Food Chem 356: 129707.
480 Lee S, Jo K, Yong HI, Choi YS, Jung S. 2021b. Comparison of the in vitro protein digestibility
481 of Protaetia brevitarsis larvae and beef loin before and after defatting. Food Chem 338:
482 128073.
483 Lee S, Jo K, Jeong HG, Choi YS, Yong HI, Jung S. 2021c. Understanding protein digestion in
484 infants and the elderly: Current in vitro digestion models. Crit Rev Food Sci Nutr 1-18.
485 Lee S, Choi YS, Jo K, Kim TK, Yong HI, Jung S. 2020a. Quality characteristics and protein
486 digestibility of Protaetia brevitarsis larvae. J Anim Sci Technol 62(5): 741
487 Lee S, Jo K, Lee HJ, Jo C, Yong HI, Choi YS, Jung S. 2020b. Increased protein digestibility of
488 beef with aging in an infant in vitro digestion model. Meat Sci 169: 108210..
ACCEPTED

489 Liu G, Xiong YL. 1996. Contribution of lipid and protein oxidation to rheological differences
490 between chicken white and red muscle myofibrillar proteins. J Agric Food Chem 44(3): 779-
491 784.
492 Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C, Carrière F, Boutrou R,
493 Corredig M, Dupont D, Dufour C, Egger L, Golding M, Karakaya S, Kirkhus B, Le Feunteun
494 S, Lesmes U, Macierzanka A, Mackie A, Marze S, McClements DJ, Ménard O, Recio I,
495 Santos CN, Singh RP, Vegarud GE, Wickham MSJ, Weitschies W, Brodkorb A. 2014. A
496 standardised static in vitro digestion method suitable for food–an international
497 consensus. Food Funct 5(6): 1113-1124.
498 Mitra B, Rinnan Å, Ruiz-Carrascal J. 2017. Tracking hydrophobicity state, aggregation
499 behaviour and structural modifications of pork proteins under the influence of assorted heat
500 treatments. Food Res Int 101: 266-273.
501 Park S, Kim N, Kim W, Moon J. 2022. The effect of Korean native chicken breed information
502 on consumer sensory evaluation and purchase behavior. Food Sci Anim Resour 42(1): 111-
503 127.
504 Pette D, Staron RS. 2000. Myosin isoforms, muscle fiber types, and transitions. Microsc Res
505 Tech 50(6): 500-509.
506 Reddish JM, Wick M, St-Pierre NR, Lilburn MS. 2005. Analysis of myosin isoform transitions
507 during growth and development in diverse chicken genotypes. Poult Sci 84(11): 1729-1734.
508 Schevzov G, Whittaker SP, Fath T, Lin JJC, Gunning PW. 2011. Tropomyosin isoforms and
509 reagents. Bioarchitecture 1(4): 135-164.
510 Stuart CA, Stone WL, Howell MEA, Brannon MF, Hall HK, Gibson AL, Stone MH. 2016.
511 Myosin content of individual human muscle fibers isolated by laser capture
512 microdissection. Am J Physiol Cell Physiol 310(5): 381-389.
513 Shin SH, Choi WS. 2022. Physiochemical properties of chicken breast sausage with red

ACCEPTED

514 singsong marc powder. Food Sci Anim Resour 42(3): 486-503.
515 Tan M, Mei J, Xie J. 2021. The formation and control of ice crystal and its impact on the quality
516 of frozen aquatic products: A review. Crystals 11(1): 68.
517 United Nations. 2019. World population prospects 2019: Highlights | multimedia library -
518 united Nations department of economic and social affairs. United Nations, NY, USA.
519 Vaskoska R, Vénien A, Ha M, White JD, Unnithan RR, Astruc T, Warner RD. 2021. Thermal
520 denaturation of proteins in the muscle fibre and connective tissue from bovine muscles
521 composed of type I (masseter) or type II (cutaneous trunci) fibres: DSC and FTIR
522 microspectroscopy study. Food Chem 343: 128544.
523 Verdiglione R, Cassandro M. 2013. Characterization of muscle fiber type in the pectoralis
524 major muscle of slow-growing local and commercial chicken strains. Poult Sci 92(9): 2433-
525 2437.
526 Vlahou A, Magni F, Mischak H, Zoidakis J. 2018. Integration of omics approaches and systems
527 biology for clinical applications. John Wiley & Sons, NJ, USA.
528 Xiong YL. 1994. Myofibrillar protein from different muscle fiber types: Implications of
529 biochemical and functional properties in meat processing. Crit Rev Food Sci Nutr 34(3):
530 293-320.
531 Young OA, Torley PJ, Reid DH. 1992. Thermal scanning rheology of myofibrillar proteins
532 from muscles of defined fibre type. Meat Sci 32(1): 45-63.
533 Zou Y, Xu P, Wu H, Zhang M, Sun Z, Sun C, Wang D, Cao J, Xu, W. 2018a. Effects of different
534 ultrasound power on physicochemical property and functional performance of chicken
535 actomyosin. Int J Biol Macromol 113: 640-647.
536 Zou X, Zhou G, Yu X, Bai, Y., Wang C, Xu X, Dai C, Li, C. 2018b. In vitro protein digestion
537 of pork cuts differ with muscle type. Food Res Int 106: 344-353.
538

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Table 1. pH, proximate composition, 10% trichloroacetic acid (TCA)-soluble α-amino groups, and the protein carbonyl content of chicken breast and thigh

Property	Breast	Thigh	SEM[1]
pH	5.81^{B}	6.49^{A}	0.005
Proximate composition			
Moisture (%)	76.13	76.57	0.265
Crude protein (%)	21.40^{A}	19.15^{B}	0.115
Crude fat (%)	1.07^{B}	3.10^{A}	0.230
Crude ash (%)	1.40^{A}	1.19^{B}	0.068
α-Amino groups (mM/g)	0.23^{B}	0.27^{A}	0.002
Protein carbonyl (nmol/mg)	4.01^{B}	5.06^{A}	0.215

[1]Standard error of the least square mean

$^{A-B}$ Different upper case letters indicate significant differences between means (P < 0.05).

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543 **Table 2. Relative content of the secondary structural components of myosin in**
544 **chicken breast and thigh**

Treatment	α-Helix	β-Sheet	β-Turn	Random coil
Breast	13.87^{B}	27.20^{A}	17.44	41.50
Thigh	14.92^{A}	26.35^{B}	17.54	41.19
SEM[1]	0.121	0.208	0.055	0.191

545 [1]Standard error of the least square mean
546 $^{A-B}$ Different upper case letters indicate significant differences between means ($p < 0.05$)
547

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548 **Figure legends**

549 **Figure 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)**
550 **electrophoretogram of myofibrillar proteins (A) and digesta (B) in chicken breast**
551 **and thigh.**

552 B, chicken breast muscle; T, chicken thigh muscle; G, gastric digesta; G + I,
553 gastrointestinal digesta.

554 **Figure 2. Intrinsic tryptophan fluorescence intensity (A) and maximum fluorescence**
555 **intensity at 328 nm (B) of myosin in chicken breast and thigh.**

556 $^{A\text{-}B}$ Different upper case letters indicate significant differences between means *(P* < 0.05).

557 **Figure 3. The α-amino group content (A) and the protein content digested under 3**
558 **kDa (B) of chicken breast and thigh.**

559 $^{A\text{-}B}$ Different upper case letters indicate significant differences between the treatments *(P*
560 < 0.05).

561 $^{a\text{-}b}$ Different lower case letters indicate significant differences between the digestive steps
562 *(P* < 0.05).

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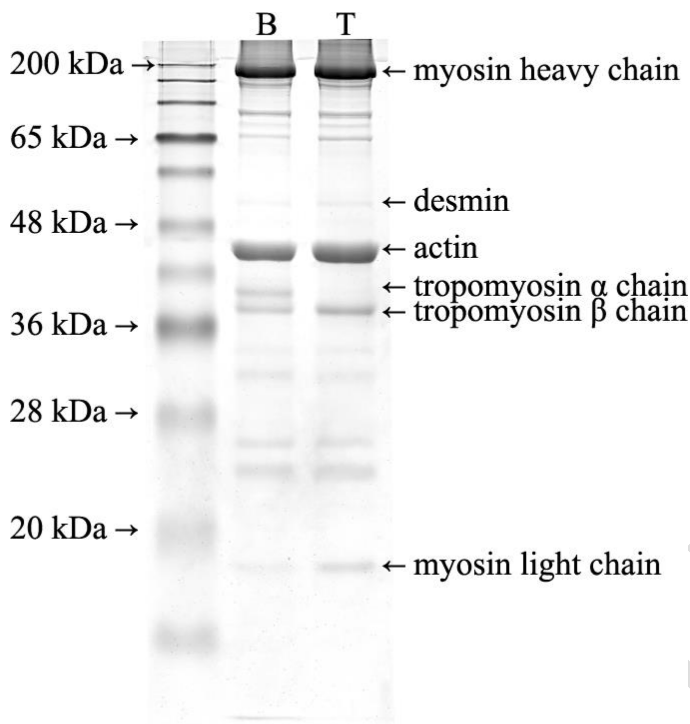
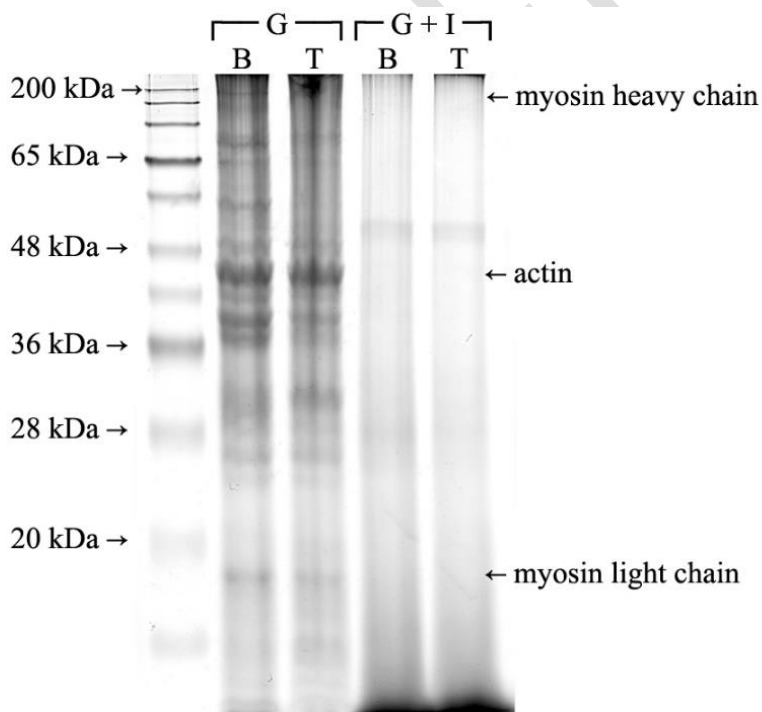
563 **(A)**



564

565 **(B)**

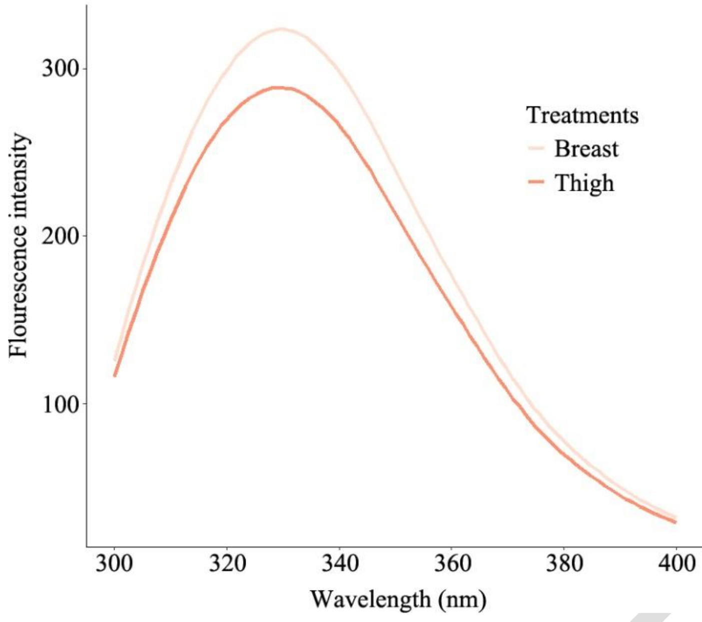


566

567 **Figure 1.**

568

569 **(A)**



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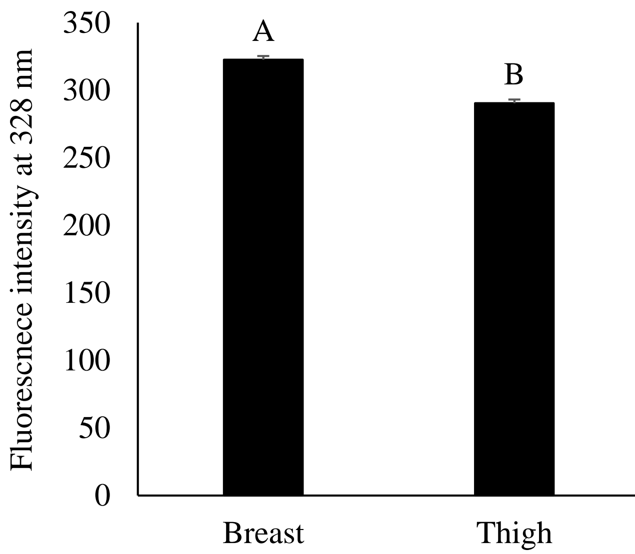
571 **(B)**

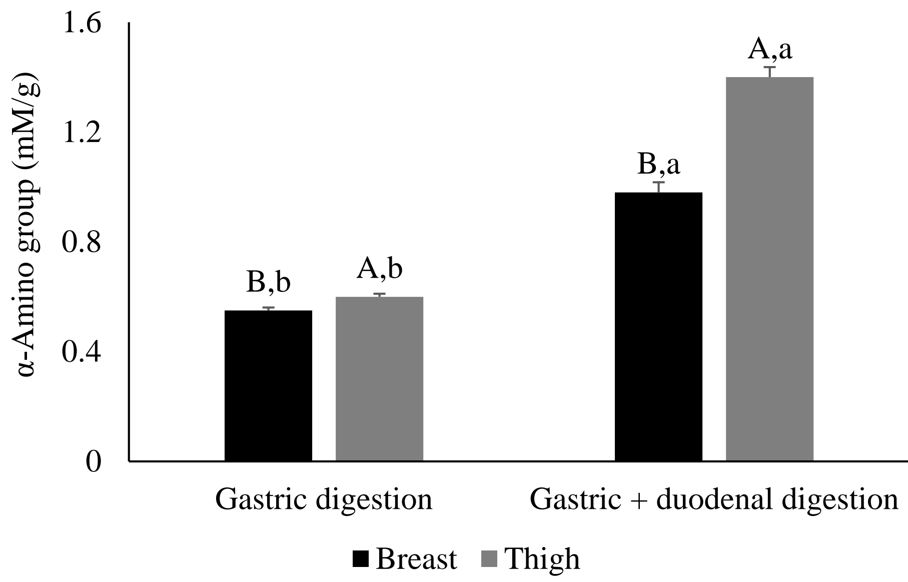


572

573 **Figure 2.**

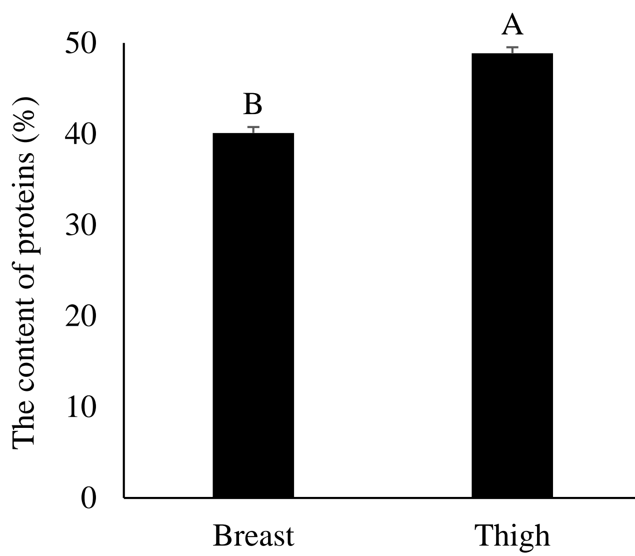
574

575 **(A)**



576

577 **(B)**



578

579 **Figure 3.**

580