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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title	Acetylation of sarcoplasmic and myofibrillar proteins were associated with ovine meat quality attributes at early postmortem
Running Title (within 10 words)	Acetylation was associated with ovine meat quality attributes postmortem
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Special remarks – if authors have additional information to inform the editorial office	
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Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This study was financially supported by the Key Program from National Natural Science Foundation of China (32030086), the Agricultural Science and Technology Innovation Program (CAAS-ASTIP-2020-IFST-03), and the Central Public-interest Scientific Institution Basal Research Fund (S2020JBKY-16) in China.
Author contributions (This field may be published.)	Conceptualization: Xin Li. Methodology: Yejun Zhang, Dequan Zhang. Investigation: Yejun Zhang. Data curation: Yejun Zhang, Xin Li, Dequan Zhang. Software: Yejun Zhang, Chi Ren, Yuqiang Bai, Muawuz Ijaz. Validation: Yejun Zhang, Muawuz Ijaz, Xu Wang, Yingxin Zhao.

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	Writing - original draft: Yejun Zhang. Writing-review & editing: Yejun Zhang, Xin Li, Dequan Zhang, Chi Ren, Yuqiang Bai, Muawuz Ijaz, Xu Wang, Yingxin Zhao. (This field must list all authors)
Ethics approval (IRB/IACUC) (This field may be published.)	This manuscript does not require IRB/IACUC approval because there are no human and animal participants.

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Abstract

The objective of this study was to examine the relationship between meat quality attributes and the changes of sarcoplasmic protein acetylation and myofibrillar protein acetylation in lamb *longissimus thoracis et lumborum* muscles at different postmortem phases. Protein acetylation, color, pH, shear force, myofibril fragmentation index and cooking loss were measured. The total level of acetylated sarcoplasmic proteins showed a negative relation with pH, a positive relation with a^*, b^* and cooking loss at the pre-rigor phase. Sarcoplasmic proteins acetylation affected postmortem pH by regulating glycolysis, which in turn affects color and cooking loss. The total level of acetylated myofibrillar proteins showed a positive relation with shear force at the pre-rigor phase. Myofibrillar proteins acetylation affected meat tenderness by regulating muscle contraction. This study indicated that acetylation played a regulatory role of meat color, water-holding capacity, and tenderization process at early postmortem.

Keywords: ovine meat, protein acetylation, color, tenderness, water-holding capacity

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24 **1. Introduction**

25 Meat quality is important to consumer satisfaction and enticing them to buy again
26 (Papanagiotou et al., 2013). Previous research indicated that the overall consumer
27 satisfaction of meat was first related to tenderness (O'Quinn et al., 2018). Meat color
28 affects consumer's purchase decision as consumers prefer bright red meat (Mancini &
29 Hunt 2005). The water-holding properties of meat is important in store and meat
30 processing (Prevolnik et al., 2010). Thus, the formation mechanism of meat tenderness,
31 water-holding capacity and color has always been the research focus in the area of meat
32 science.

33 A series of physiological and biochemical changes occur after the animals are
34 slaughtered, of which rigor mortis is the most significant change. As glycolysis is the
35 source of ATP in muscle cells after slaughter, glycolysis determines rigor mortis. Early
36 glycolysis after slaughter is very important to meat quality, too fast or too slow
37 glycolysis will cause heterogeneous meat and bring losses to the meat industry (Lawrie
38 & Ledward 2006). As the time after slaughter prolonged, muscle undergoes the process
39 of rigor mortis and aging, meat quality attributes continuously change during this
40 process. Based on the development of rigor mortis, meat can be divided into hot fresh
41 meat (pre-rigor) and aged meat (post-rigor). Recent studies have shown that the hot
42 fresh meat and aged meat has different meat quality characteristics. The hot fresh meat
43 has lower cooking loss in comparison with aged meat, but the tenderness was better in
44 aged meat (Xiao et al., 2020). Hence, to explore the formation mechanism of meat
45 quality characteristics at different postmortem phases and enrich meat quality
46 regulatory theory is needed.

47 Many studies have been carried out on mechanism of meat quality development
48 postmortem, but there are still issues of large variation in tenderness, color deterioration,
49 and drip in the meat industry, indicating that the reason for meat quality difference at
50 different postmortem phases has not been fully understood and the regulatory
51 mechanisms also have not been fully elucidated (Bhat et al., 2018; Mancini & Hunt

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52 2005).

53 There have been some reports on the mechanism of protein phosphorylation affects
54 meat quality during postmortem storage (Wang et al., 2019; Li et al., 2018). Acetylation
55 is also a crucial modification of protein lysine ε-amino group that acetyltransferase
56 catalyzes the transfer of acetyl group from acetyl coenzyme A (Ac-CoA) to lysine. The
57 positively charged acetyl group changes the structure of the lysine side chain to regulate
58 protein homeostasis, intracellular signaling and biomolecular interactions (Narita et al.,
59 2018). Jiang et al. (2019) suggested that the dynamic protein acetylation was related to
60 muscle postmortem changes that may affect meat color, drip loss, pH and cooking yield
61 in pork. Studies have found that protein acetylation positively regulated the postmortem
62 glycolysis of muscle (Li et al., 2016; Li et al., 2017b). Furthermore, a proteomic study
63 suggested that pre-slaughter handling may regulate meat color, tenderization, water
64 distribution, and pH via acetylation/deacetylation of glycolysis enzymes (Zhou et al.,
65 2019). Altogether, protein acetylation may involve in the transformation from muscle
66 to meat and affect meat quality formation at different postmortem phases.

67 Hence, this experiment dedicated to investigate the effects of protein acetylation on
68 meat quality at different postmortem phases (pre-rigor, rigor mortis and post-rigor) in
69 terms of myofibrillar and sarcoplasmic proteins that are the two types of proteins with
70 the highest proportion in muscle tissue. The relationship between protein acetylation
71 (sarcoplasmic proteins and myofibrillar proteins) and meat quality attributes
72 (tenderness, color, and water-holding capacity) in ovine *longissimus thoracis et*
73 *lumborum* (LTL) muscles at different postmortem phases were investigated to provide
74 new insight on the variation of meat quality at different postmortem phases.

75 2. Materials and methods

76 2.1 Sampling and treatments

77 Ten carcasses of crossbred sheep (fat tailed Han × local sheep) were randomly collected
78 from a local slaughterhouse. These sheep were 8 months old and had the same feeding
79 system, batch, genetic background, sex and pre-mortem treatment. The butchery of

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80 sheep was carried out in a morning using standard practices of the slaughterhouse. The
81 mean carcass weight was 27.8 kg (range 27.0-28.6 kg). The LTL muscles were collected
82 within 30 min after slaughtering. Superficial fascia and fat were removed before
83 wrapped the LTL muscles with oxygen-permeable membrane and stored at 4℃. At 1 h
84 postmortem, two pieces of LTL muscles from the left side weighed approximately 65 g
85 were cut for pH and color measurement respectively. Afterwards, the two pieces of meat
86 were wrapped and stored at 4℃ again for the pH and color measurement at 1 h, 6 h, 12
87 h, 1 d, 2 d, 3 d, 5 d and 7 d postmortem. The remaining left-side and right-side LTL
88 muscle were collected at the same postmortem time points that some used for shear
89 force and cooking loss measurements, others were stored at -80°C for myofibril
90 fragmentation index (MFI) and protein acetylation analysis respectively.

91 **2.2 Meat quality attributes**

92 **2.2.1 pH**

93 The pH meter (testo 205, Lenzkirch, Germany) was performed a 2-point calibration in
94 buffers at pH value of 4.00 and 7.00 before measurement. The average pH value was
95 calculated by taking the mean of three separate positions of each LTL muscle samples.

96 **2.2.2 Color**

97 The determination of meat color was following the published literature (Li et al., 2017a)
98 using CM-600D colorimeter (Konica Minolta Holdings Inc., Tokyo, Japan). For each
99 sample, four random points were selected for color measurement. The color parameters
100 including lightness (L^*), redness (a^*) and yellowness (b^*) were read directly from the
101 instrument and averaged for statistical analysis.

102 **2.2.3 Myoglobin redox forms**

103 The reflectivity values from 360 nm to 740 nm were obtained from the colorimeter for
104 the calculation of reflectance values at 474 nm, 525 nm, and 572 nm (Li et al., 2017a).
105 The Kubelka–Munk K/S values were calculated by $K/S = (1 - R)^2/(2 R)$, R = %
106 reflectance. The relative content of myoglobin redox forms was calculated by the
107 equation deoxymyoglobin (DeoxyMb %) = [1.5 − (K/S474)/(K/S525)], metmyoglobin

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108 (MetMb %) = [2 − (K/S572)/(K/S525)] and oxymyoglobin (OxyMb %) = [1 −
109 (K/S610)/(K/S525)] (AMSA, 2012; Li et al., 2018).

110 **2.2.4 Myofibril fragmentation index**

111 Myofibril fragmentation index was measured according to Wang et al. (2018) but with
112 minor changes: (1) muscles were homogenized three times for 30 s using dispersing
113 machine (IKA Labortechnik, Staufen, Germany), (2) half MFI buffer was used for the
114 second resuspending; and (3) the suspension of myofibrils was diluted five times before
115 protein concentration determination.

116 **2.2.5 Cooking loss and shear force**

117 Cooking loss and shear force were measured by the methods described by Hopkins et
118 al. (2010) and Holman et al. (2015). A certain weight of muscle (73.5 ± 12.1 g) was
119 collected from the LTL for cooking loss measurement at each postmortem time point
120 and weighed as m_1. The samples were soaked in 71°C water using cooking bags. Thirty-
121 five minutes later, the samples were moved into cold running water for 30 min.
122 Afterwards, drying the meat pieces with filter paper and reweighed as m_2, cooking loss
123 was calculated by $(m_1 - m_2)/m_1 \times 100\%$.

124 The cooked blocks were stored at 4–5°C overnight to measure shear force. Twelve
125 cuboidal strips (length 4 cm, width 1 cm, height 1 cm) were cut off parallel to the fiber
126 orientation. Afterwards, the V-slot blade (TA. XT plus® texture analyser, UK) was set
127 to 1 mm s^{-1} crosshead speed to measure the peak force (Newtons) required to cut the
128 cuboidal strips.

129 **2.3 Protein acetylation**

130 The determination of the total level of acetylated sarcoplasmic and myofibrillar proteins
131 was done according to Li et al. (2016). Briefly, the concentration of sarcoplasmic
132 protein and myofibrillar proteins was measured by BCA (bicinchoninic acid) assay kit,
133 and then take a certain amount of protein solution to run electrophoresis. Finally, the
134 relative content of the acetylated proteins in the total proteins is determined by the
135 acetylated lysine antibody using western blotting, which is recorded as the total level

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136 of acetylated proteins. The detailed method is as follows.

137 **2.3.1 Proteins extraction**

138 Frozen samples (1 g) were homogenized (3 × 15 s with 15 s break on ice between bursts)
139 in 6 mL pre-chilled extraction buffer (0.6057 g Tris, 0.0771 g DTT, pH 8.3, one tablet
140 protease inhibitor per 50 ml) by dispersing machine. The sarcoplasmic and myofibril
141 proteins were separated by centrifugation at 10,000g for 30 min (4°C). The
142 sarcoplasmic protein concentration of supernatant was measured using the BCA method
143 and then adjusted to 4 mg mL^{-1}. Dissolve the pellets (myofibrillar proteins) with 15 ml
144 5% SDS (sodium dodecyl sulfate) solution firstly, and then heated for 20 min at 80°C.
145 Afterwards, the myofibrillar protein concentration was adjusted to 4 mg mL^{-1}. Equal
146 volume of loading buffer was added into the diluted supernatant and pellet solution.
147 Finally, the solution was heated in 100°C for 10 min and then refrigerated at -80°C until
148 gel electrophoresis.

149 **2.3.2 Western blotting**

150 Western blotting analyses of acetylated proteins were performed according to the
151 standard procedure. Ten microliters sample was separated on 12% Mini-PROTEIN
152 TGX Precast Gels. Set the voltage at 70 V to pass the sample through the concentrated
153 gel, and then maintain the voltage at 110V to pass the sample through the separation
154 gel. Proteins on gels were transferred to nitrocellulose membrane at 100 V for 200 min.
155 After blocked for 1 h at room temperature, the membrane was incubated with first
156 antibody (PTM Bio, Hang Zhou, China) overnight at 4°C, and incubated with second
157 antibody (CST, MA, USA) for 1 h or so at room temperature. Acetylated proteins were
158 visualized using enhanced chemiluminescence (ECL) kit.

159 **2.4 Statistical analysis**

160 One-way ANOVA was performed for comparisons of postmortem time points by SPSS
161 Statistic 21.0. The principal component analysis (PCA) was performed by Origin 2018
162 software (OriginLab, USA). The significance was determined by the Duncan's Test at
163 the 5% confidence level. The results were expressed as average and standard deviation.

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3. Results

3.1 pH

As shown in Table 1, pH declined significantly from 1 h to 12 h postmortem ($p<0.05$) and then remained stable until 3 d postmortem. The pH values decreased significantly again ($p<0.05$) after 3 d postmortem, and reached the minimum value on 5 d postmortem, which increased significantly from 5 d to 7 d postmortem ($p<0.05$). The pH value of 7 d still lower than that of 6 h postmortem ($p<0.05$).

3.2 Color

The L^*, a^* and b^* values of LTL muscles stored at 4°C were shown in Table 1. The L^* and b^* increased from 1 h to 2 d postmortem, moreover, the L^* and b^* of 2 d were higher than 1 h, 6 h and 12 h ($p<0.05$). The L^* decreased significantly from 3 d to 7 d postmortem ($p<0.05$). The b^* decreased significantly from 3 d to 5 d postmortem ($p<0.05$). Similarly, the a^* increased significantly from 1 h to 12 h postmortem and reached the maximum value at 12 h postmortem ($p<0.05$). The a^* value decreased from 2 d to 7 d postmortem, which was lower on 7 d postmortem than that on 2 d, 3 d and 5 d postmortem ($p<0.05$).

3.3 Myoglobin redox forms

The percentage of DeoxyMb, MetMb and OxyMb were shown in Fig. 1. The DeoxyMb decreased from 6 h to 1 d and increased from 3 d to 5 d postmortem ($p<0.05$). The relative content of MetMb decreased significantly from 1 h to 6 h postmortem ($p<0.05$). The MetMb increased from 1 d to 3 d postmortem, and reached the maximum on 3 d ($p<0.05$). Furthermore, the MetMb content decreased significantly again from 3 d to 7 d ($p<0.05$). The relative content of OxyMb increased significantly from 1 h to 12 h postmortem ($p<0.05$). It was noted that the OxyMb of 7 d postmortem were lower than all other time points except 1 h postmortem ($p<0.05$).

3.4 Myofibril fragmentation index

As shown in Table 1, the myofibril fragmentation index (MFI) of LTL muscles always increased within 7 d postmortem ($p<0.05$). MFI at 5 d and 7 d postmortem were higher

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192 than that at all other timepoints ($p<0.05$).

193 **3.5 Shear force**

194 The shear force did not change from 1 h to 12 h postmortem (Table 1, $p>0.05$).
195 Furthermore, the shear force reached the maximum value on 1 d postmortem, which
196 was higher than that at all other timepoints ($p<0.05$). It was noted that no significant
197 difference in shear force was observed between 2 d and 3 d, 5 d and 7 d postmortem
198 ($p>0.05$). According to the change of shear force, the pre-rigor period was 1 h-12 h
199 postmortem, the process of rigor mortis was 12 h-2 d postmortem, whereas the stage of
200 the post-rigor was 2 d-7 d postmortem.

201 **3.6 Cooking loss**

202 The cooking loss of 1 h, 6 h, 12 h and 1 d was lower than 3 d postmortem (Table 1,
203 $p<0.05$). No significant difference in cooking loss was observed between 3 d and 5 d,
204 5 d and 7 d postmortem ($p>0.05$), but the cooking loss of 3 d postmortem was higher
205 than that on 7 d postmortem ($p<0.05$).

206 **3.7 Total level of acetylated sarcoplasmic proteins and myofibrillar proteins**

207 The acetylated sarcoplasmic proteins (Fig. 2A) and myofibrillar proteins (Fig. 3A) were
208 visualized by western blotting. The total level of acetylated sarcoplasmic proteins and
209 myofibrillar proteins both showed a trend of first increased and then decreased (Fig. 2B,
210 Fig. 3B). The total level of acetylated sarcoplasmic proteins was significantly higher on
211 2 d postmortem than that at 1 h and 7 d postmortem ($p<0.05$). The total level of
212 acetylated myofibrillar proteins was significantly higher on 1 d postmortem than that at
213 1 h, 5 d and 7 d postmortem ($p<0.05$).

214 **3.8 Multivariate statistical analysis**

215 The relationship between the total level of sarcoplasmic and myofibrillar protein
216 acetylation and pH, color, cooking loss, shear force, and MFI at different storage stages
217 were investigated by PCA. The PCA applied to the data matrix from 1 h to 12 h
218 postmortem (Fig. 4A) showed that the cosine angles among total level of acetylated
219 sarcoplasmic proteins and cooking loss, L^*, a^*, b^*, OxyMb were less than 90°. The total

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220 level of acetylated sarcoplasmic proteins showed a positive correlation with L^*, a^*, b^*,
221 OxyMb, and cooking loss, whereas these variables were negatively correlated with pH.
222 The total level of acetylated myofibrillar proteins were positively correlated with shear
223 force and MFI.
224 The PCA applied to the data matrix from 12 h to 2 d postmortem (Fig. 4B) suggested
225 that the total level of acetylated sarcoplasmic proteins showed a positive correlation
226 with cooking loss, L^*, b^*. Whereas these variables were negatively correlated with pH.
227 It was noted that the a^* was positively correlated with pH and negatively correlated with
228 the total level of acetylated sarcoplasmic proteins. Furthermore, the total level of
229 acetylated myofibrillar proteins were positively correlated with shear force.
230 The PCA applied to the data matrix from 2 d to 7 d postmortem (Fig. 4C) suggested
231 that the total level of acetylated sarcoplasmic proteins was positively correlated with
232 cooking loss, L^*, a^*, b^*. Whereas pH was negatively correlated with these variables. In
233 addition, the total level of acetylated myofibrillar proteins were positively correlated
234 with shear force and the MFI was negatively correlated with shear force.

235 **4. Discussion**

236 **4.1 Effect of postmortem time on meat quality attributes**

237 The pH decreased gradually within 12 h postmortem indicating that the glycolysis has
238 finished at the pre-rigor phase. The value of L^* increased during the pre-rigor period
239 and then decreased, which could be explained by the firstly increased and then
240 decreased tendency of drip loss. Changes in water-holding capacity result in changes in
241 meat surface moisture, which in turn affects L^* (Mungure et al., 2016). The value of a^*
242 increased gradually because of the increased OxyMb content from the period of pre-
243 rigor to rigor mortis. However, with prolonged storage time, Oxidation of OxyMb to
244 MetMb and lipid oxidation gradually increased, therefore the value of a^* declined until
245 7 d postmortem (Bekhit et al., 2007). Similarly, the value of b^* showed a firstly
246 increased and then decreased tendency, this may be interpreted as the changed ratio of
247 OxyMb/myoglobin postmortem (Lindahl et al., 2001). MFI is inversely related to shear

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248 force and is usually used to indicate the tenderness of meat (Culler et al., 1978; Olson
249 et al., 1976). The myofibril fragmentation index increased during the whole postmortem
250 storage, which could be interpreted as the proteolytic breakdown of myofibrillar
251 proteins (Hopkins et al., 2000). It can be inferred from the Table 1 that the meat reached
252 maximum rigor period on 1 d as the shear force reached the maximum value.
253 Afterwards, as the myofibrillar protein breakdown, and the muscle ultrastructure
254 destruction, the muscle reached post-rigor period and the shear force decreased
255 gradually, which was similar with previous results (Wheeler & Koohmaraie 1994). With
256 the change of pre-rigor to rigor mortis, the thick filaments combine with thin filaments
257 to form an irreversible cross bridge, which results in the contraction of muscle spatial
258 structure and increase in cooking loss consequently. From rigor mortis to post-rigor, the
259 protein degradation and disruption of muscle integrity led to an increase in cooking loss
260 (Abdullah & Qudsieh 2009).

261 **4.2 Effect of postmortem time on protein acetylation**

262 Protein acetylation is one of the major post-translational modifications in both
263 prokaryotes and eukaryotes (Drazic et al., 2016). The changes in the acetylation of
264 sarcoplasmic and myofibrillar proteins in postmortem muscles was investigated in this
265 study. Since acetyl coenzyme A (Ac-CoA) was the main acetyl donor, it was proposed
266 that the changes in total level of acetylated sarcoplasmic and myofibrillar proteins in
267 postmortem muscles may be mainly affected by changes in Ac-CoA content (Kato 1978;
268 Poleti et al., 2018; Ricny & Tucek 1980). Termination of blood supply in muscle tissue
269 after slaughter may lead to an increase in the content of Ac-CoA at the pre-rigor phase,
270 and then as the cessation of metabolism, the content of Ac-CoA decreased (Kato 1978;
271 Poleti et al., 2018; Ricny & Tucek 1980). Therefore, the protein acetylation increased
272 at the pre-rigor phase and then decreased with the exhaustion of Ac-CoA in the LTL
273 muscles.

274 **4.3 Comparison of relationship between protein acetylation and meat quality**
275 **in three different postmortem periods**

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The pH value and total level of acetylated sarcoplasmic proteins showed a strong negative correlation at the pre-rigor and the rigor mortis phase, but weakened at the post-rigor phase. Previous research showed that protein acetylation can increase the activity and stability of glycolytic enzymes, thereby affecting the glycolysis rate postmortem (Xiong & Guan 2012; Li et al., 2017b). For sarcoplasmic proteins, many acetylated glycolysis and glycogen metabolism enzymes include glycogen phosphorylase, pyruvate kinase and glyceraldehyde-3-phosphate dehydrogenase were identified, which showed a decreased acetylation level within 24 hours postmortem (Jiang et al., 2019; Li et al., 2017b). Glycogen phosphorylase, and phosphofructokinase are glycometabolic rate-limiting enzymes in glycolysis. Thus, protein acetylation positively regulated the glycolysis process and accelerate the pH decline rate at early postmortem. And with the decrease of glycolytic enzyme acetylation level, its promotion of the glycolytic process weakens. In addition, research showed that pH was related to all other meat attributes and could reflect the overall quality of meat (Kang et al., 2019). In summary, sarcoplasmic protein acetylation negatively regulated the pH value postmortem and be associated with the overall meat quality by controlling glycolysis at early postmortem.

Meat color was governed by the interactions between myoglobin and various external and internal factors (Mancini & Hunt 2005; Suman & Joseph 2013). In this study, the total level of acetylated sarcoplasmic proteins showed a positive correlation with a^*, b^* and OxyMb, a negative correlation with DeoxyMb at the pre-rigor phase. The reason could be that myoglobin acetylation increase its oxygen binding capacity and oxygen content was higher in the early postmortem, which in turn leads to an increase in a^* (Suman & Joseph 2013; Jiang et al., 2019; Lindahl et al., 2001). The positive relationship between total level of acetylated sarcoplasmic proteins and b^* value at the pre-rigor phase possibly because both of a^* and b^* are related to myoglobin forms (Suman & Joseph 2013). The more myoglobin oxygenation resulted in more redness and higher ratio of OxyMb /myoglobin, which will result in more yellowness (Lindahl

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304 et al., 2001). However, although acetylation increased its oxygen binding capacity of
305 myoglobin, myoglobin was mainly oxidized to produce MetMb due to the decrease of
306 oxygen content with the extension of postmortem time (Suman & Joseph 2013). Thus,
307 a^* showed a strong negative correlation with the total level of acetylated sarcoplasmic
308 proteins and MetMb, and a weak negative correlation with OxyMb at the rigor mortis
309 phase. The total level of acetylated sarcoplasmic proteins decreased with the exhaustion
310 of Ac-CoA at the post-rigor phase, thus the oxygen binding capacity of myoglobin
311 decreased. As a result, a^* and b^* value decreased due to the gradually decreased OxyMb
312 content and increased lipid oxidation (Jiang et al., 2019; Bekhit et al., 2007). Thus,
313 $a^*$and b^* value positively correlated with the total level of acetylated sarcoplasmic
314 proteins at the post-rigor phase. In summary, sarcoplasmic protein acetylation improved
315 meat color by increasing myoglobin oxygen binding capacity at early postmortem. At
316 the same time, the values of a^* and b^* were negatively correlated with pH at the pre-
317 rigor phase. The reason could be that the high pH values increased the meat surface
318 oxygen consumption rate, which inhibited the formation of OxyMb cladding (Aalhus
319 et al., 2001; Simmons et al., 2008). Therefore, sarcoplasmic protein acetylation
320 regulated the a^* and b^* of LTL muscles by controlling the glycolysis and myoglobin
321 function at early postmortem.
322 Cooking loss was positively correlated with the total level of acetylated sarcoplasmic
323 proteins throughout the seven days postmortem. This may be because acetylation
324 changes the protein charge state, and the internal hydrophobic groups such as sulfhydryl
325 group is exposed. The exposed hydrophobic groups increase the hydrophobicity of the
326 protein, and then leads to the easy aggregation of the protein and the formation of
327 precipitation (Ren et al., 2015; Zuo et al., 2018). Precipitation of sarcoplasmic proteins
328 on myofibrils can reduce the electrostatic repulsion between filaments, leading to
329 increased moisture loss (Eikelenboom & Smulders 1986). Furthermore, previous
330 research showed that the decrease in muscle water-holding capacity was related to
331 muscle contraction, degradation and changes of temperature and pH postmortem

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(Lawrie & Ledward 2006). pH was associated with the myofibrillar protein breakdown and actomyosin dissociation during postmortem storage (Starkey et al., 2016; Wu et al., 2014). As protein acetylation has been proved to be involved in the energy metabolism postmortem, sarcoplasmic protein acetylation affected cooking loss by changing pH in muscle. Moreover, the L^* showed a positive correlation with cooking loss at rigor mortis and post-rigor. This could be explained by the kept increasing water-holding capacity of fresh meat after slaughtering, causing moisture to leak out on the surface of the meat, increasing L^* (Mungure et al., 2016).

Tenderness is recognized as the most critical meat quality attribute as variation of tenderness is the most common cause of unsatisfied meat (Jeremiah 2009). Several acetylated myofibrillar proteins involved in rigor mortis had been identified, which indicated that protein acetylation may affect the postmortem tenderization process (Foster et al., 2013; Jiang et al., 2019). In all the three different rigor periods, shear force showed a positive correlation with the total level of acetylated myofibrillar proteins, while the correlations at the pre- and post-rigor phase were higher than that at the rigor mortis phase. Abe et al. (2000) reported that actin acetylation facilitated its weak interaction with myosin. Viswanathan et al. (2015) reported that the acetylation of actin could alter electrostatic associations between tropomyosin and myosin, attenuate tropomyosin's inhibition of binding of actin and myosin, and thereby enhances actomyosin associations. Furthermore, the acetylation of myosin could decrease the Michaelis constant (K_m, the concentration of substrate at which the reaction takes place at one half its maximum rate) of the actin-activated ATPase activity and increased the interaction with actin (Samant et al., 2015). At pre-rigor phase, in addition to the increasing actomyosin content due to the reduction of ATP, the acetylation of myofibrillar protein also contributed to the inhibition of actomyosin dissociation. While at post-rigor phase, as the total level of acetylated myofibrillar proteins decreases, its inhibitory effect on actomyosin dissociation is weakened. Thus, the total level of acetylated myofibrillar proteins showed a positive correlation with shear force at the

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360 pre- and post-rigor phase. The correlations between shear force and the total level of
361 acetylated myofibrillar proteins at the rigor mortis phase was lower than that at the pre-
362 and post-rigor phase. Probably because the binding of myosin and actin has reached the
363 maximum at rigor mortis phase, the rigidity of muscle was in a slowly changing state.
364 Shear force was positively correlated with MFI at the pre-rigor phase, negatively
365 correlated with MFI at the rigor mortis and post-rigor phase. This could be because the
366 increase in MFI has a smaller effect on muscle tenderization than the increase in shear
367 force caused by the combination of myosin and actin to form an irreversible cross
368 bridge at the pre-rigor phase (Culler et al., 1978). In summary, myofibrillar protein
369 acetylation negatively regulated tenderness by inhibiting actomyosin dissociation,
370 especially in the early and late postmortem.

371 **5. Conclusion**

372 The regulatory effect of protein acetylation on meat quality is mainly reflected in the
373 early postmortem (1 h-12 h). In the early postmortem period, acetylation of
374 sarcoplasmic protein negatively regulates pH and water-holding capacity, and
375 positively regulates meat redness; acetylation of myofibrillar protein negatively
376 regulates tenderness.

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495 **Table 1**

496 Meat quality attributes of ovine *longissimus thoracis et lumborum* (LTL) muscles stored

497 at 4°C for 7 days postmortem.

	Postmortem times								P-value
	1 h	6 h	12 h	1 d	2 d	3 d	5 d	7 d	
pH	6.07 ± 0.25^{a}	5.92 ± 0.21^{b}	5.74 ± 0.14^{cd}	5.76 ± 0.11^{cd}	5.70 ± 0.13^{cd}	5.67 ± 0.19^{d}	5.51 ± 0.20^{e}	5.81 ± 0.14^{c}	< 0.001
L*	37.02 ± 1.28^{d}	37.46 ± 3.54^{d}	38.85 ± 1.90^{c}	42.72 ± 1.79^{a}	43.76 ± 1.19^{a}	43.57 ± 1.46^{a}	40.65 ± 2.27^{b}	37.69 ± 2.67^{d}	< 0.001
a*	8.26 ± 0.82^{d}	13.18 ± 1.11^{b}	15.10 ± 1.14^{a}	15.08 ± 1.46^{a}	14.55 ± 1.26^{a}	13.40 ± 1.29^{b}	13.06 ± 0.96^{b}	11.45 ± 1.92^{c}	< 0.001
b*	5.72 ± 0.86^{d}	10.70 ± 1.52^{c}	12.29 ± 1.26^{b}	15.01 ± 0.88^{a}	15.73 ± 1.42^{a}	15.59 ± 1.02^{a}	12.87 ± 1.78^{b}	10.43 ± 1.10^{b}	< 0.001
MFI	36.02 ± 7.75^{e}	46.11 ± 10.94^{d}	50.75 ± 11.86^{d}	51.98 ± 10.09^{d}	62.72 ± 9.25^{c}	76.01 ± 7.99^{b}	84.97 ± 7.69^{a}	89.03 ± 6.11^{a}	< 0.001
Shear force (N)	61.23 ± 5.46^{bc}	66.48 ± 8.42^{abc}	69.11 ± 3.93^{ab}	72.71 ± 7.50^{a}	62.73 ± 15.13^{bc}	57.01 ± 13.30^{c}	42.15 ± 12.66^{d}	36.60 ± 10.20^{d}	< 0.001
Cooking loss (%)	11.00 ± 3.44^{d}	13.85 ± 4.32^{cd}	13.50 ± 2.71^{d}	16.71 ± 3.72^{bc}	19.13 ± 3.46^{ab}	21.10 ± 2.91^{a}	18.34 ± 3.08^{ab}	17.66 ± 3.33^{b}	< 0.001

498 The results were shown as means and standard deviation. Data with different letters in

499 a row are significantly different ($p < 0.05$).

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501 **Figure captions**

502 **Fig. 1.** Myoglobin redox forms of ovine *longissimus thoracis et lumborum* (LTL)
503 muscles stored at 4°C for 7 days. a–e: different letters indicate significant difference (p
504 < 0.05) between storage times. The results were shown as means and standard deviation.

505 **Fig. 2.** The total level of acetylated sarcoplasmic proteins of ovine muscle stored at 4°C
506 for 7 d postmortem. Western blotting of acetylated sarcoplasmic proteins (A).
507 Quantification of the acetylated sarcoplasmic proteins (B). a-b: Different letters are
508 significantly different at different postmortem time ($p < 0.05$). St: Standard. The results
509 were shown as means and standard deviation.

510 **Fig. 3.** The total level of acetylated myofibrillar proteins of ovine muscle stored at 4°C
511 for 7 d postmortem. Western blotting of acetylated myofibrillar proteins (A).
512 Quantification of the acetylated myofibrillar proteins (B). a-b: Different letters are
513 significantly different at different postmortem time ($p < 0.05$). St: Standard. The results
514 were shown as means and standard deviation.

515 **Fig. 4.** Biplot for the first two principal components (PC1 and PC2) for the 12 variables
516 from 1 h to 12 h (A), 12 h to 2 d (B) and 2 d to 7 d (C) postmortem. The location of the
517 variables in the multivariate space was according to their component loadings that
518 represents the correlations between the variable and the component. TASP: total level
519 of acetylated sarcoplasmic proteins. TAMP: total level of acetylated myofibrillar
520 proteins.

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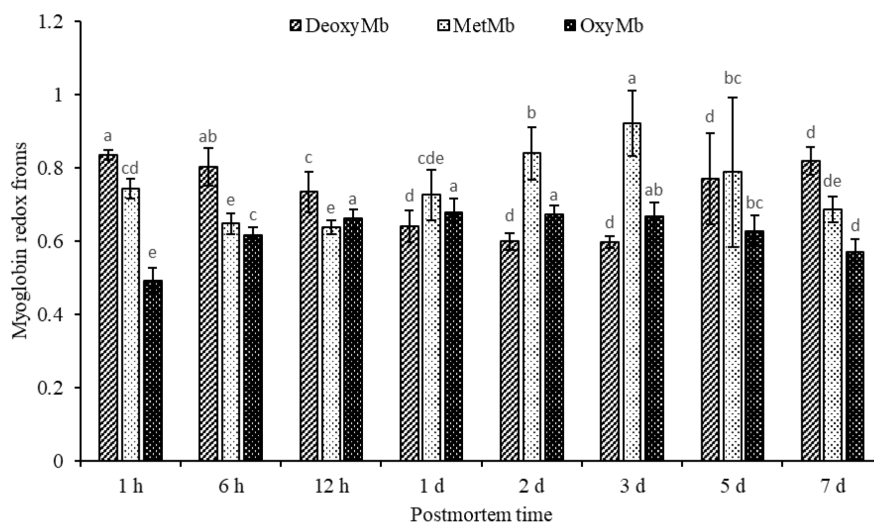
522 **Figure 1**



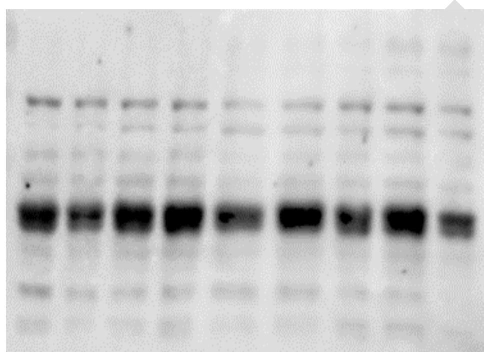
523

524 **Figure 2**

525

A

526



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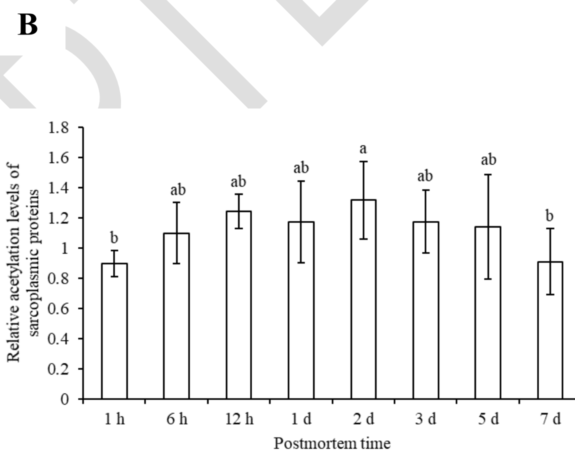

527

528 **Figure 3**

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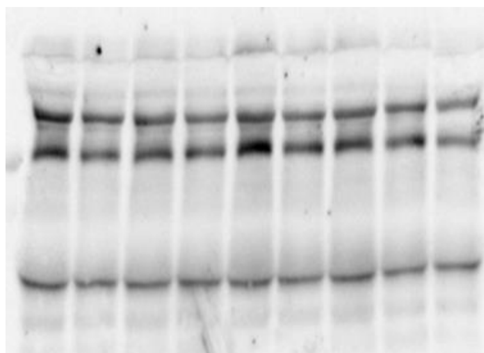
A

530



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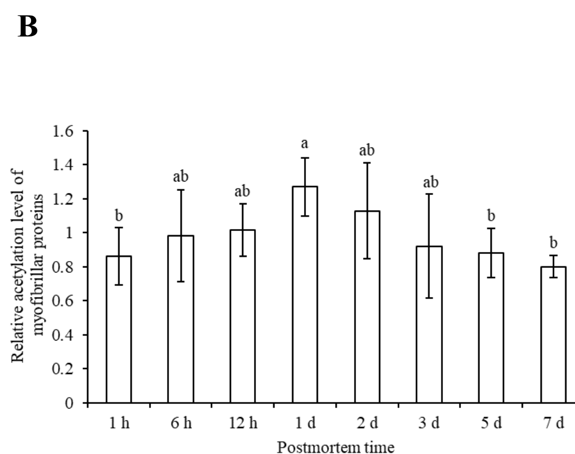

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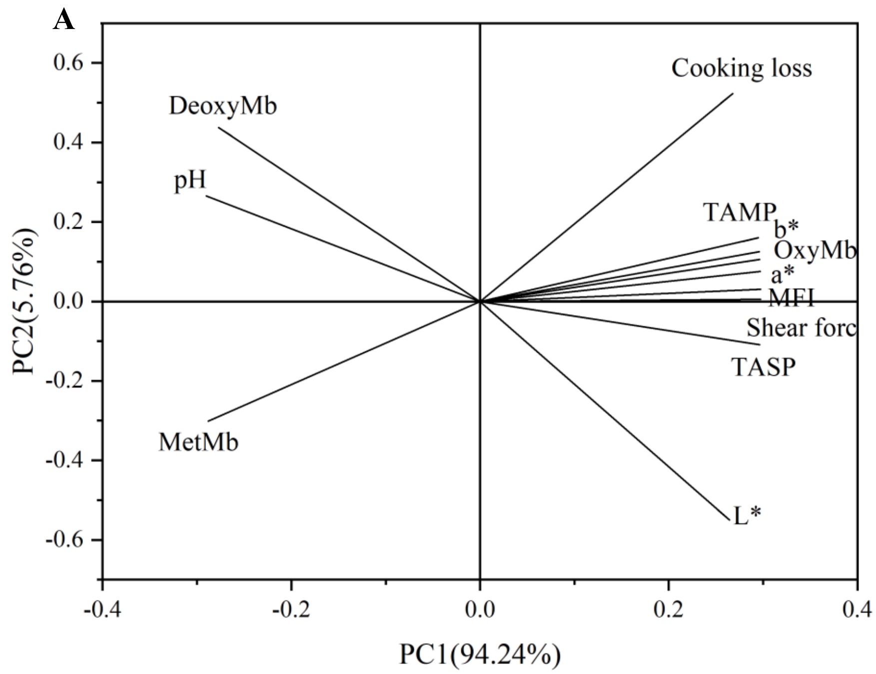
533 **Figure 4**



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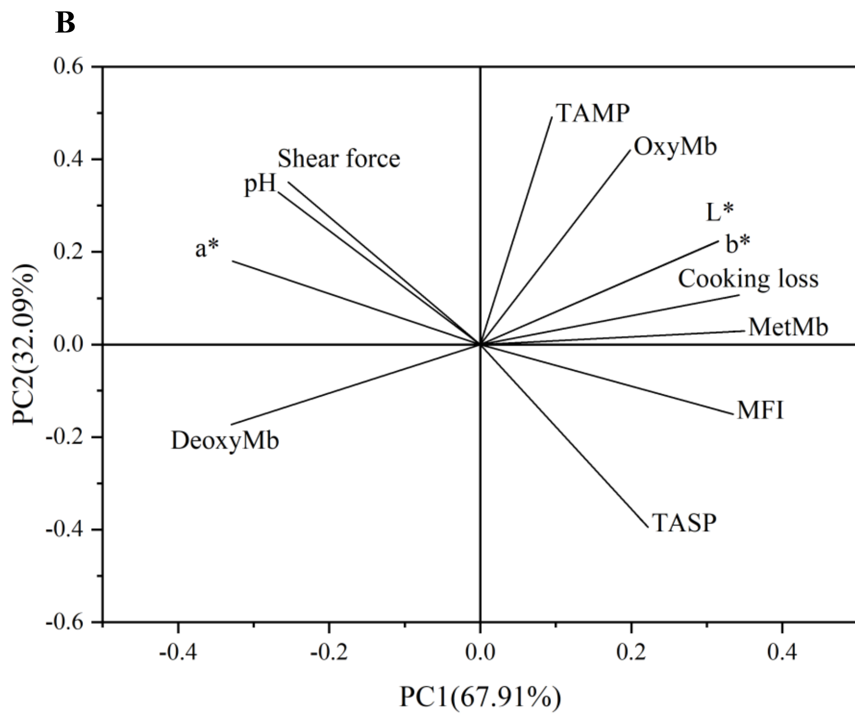

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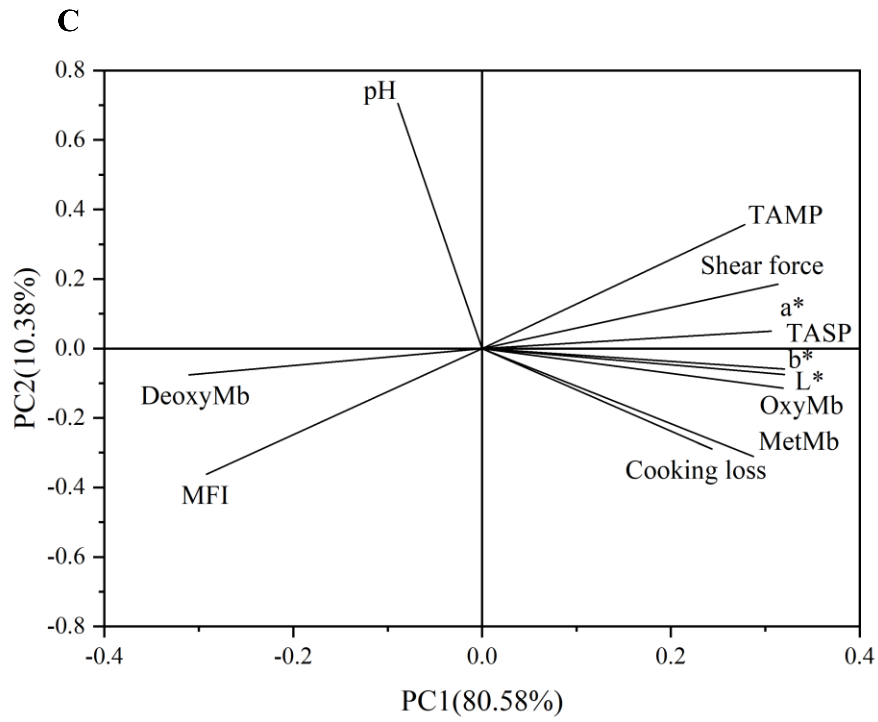

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