Proteomic Analysis of Goat *Longissimus dorsi* Muscles with Different Drip Loss Values Related to Meat Quality Traits

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Abstract *Longissimus dorsi* muscles from 3 goat species were assigned to high and low drip loss groups. Physio-chemical properties, sarcomere length, and proteome profiles were investigated. The high drip loss group had lower pH, higher brightness, and higher shear force values, and shorter sarcomere lengths than the low drip loss group. 22 differential proteins were identified between high and low loss groups. α -Enolase, NADH dehydrogenase, pyruvate dehydrogenase E1, HSP27, superoxide dismutase, peroxiredoxin-2, myosin, and the myosin light chain were among these proteins, which were metabolic enzymes, stress response factors, and structural proteins that affected glycolysis, oxidation, and muscle contraction. Drip loss was probably produced via proteins involved in glycolysis, oxidation, and muscle contraction.

Keywords: goat, drip loss, metabolic enzyme, stress response factor, structural protein

Introduction

China is the world's biggest producer and consumer of lamb and goat meat with a production level of 4.28 million tons in 2014. The total drip loss in lamb and goat production reaches 150,000 tons a year in China, which is equal to an economic loss of 8 billion Yuan. The yield of goat is slightly lower than lamb, with a ratio of 45 to 55%, but the goat drip loss is commonly 0–2% higher than for lamb. Drip loss is a key indicator of the water holding capacity (WHC) of meat, which is influenced by genetics (the halothane and RN genes), feed composition, and pre-slaughter and postmortem handling (1). At a molecular level, the WHC is related to protein denaturation (2), protein oxidation (3), and actomyosin interaction (muscle contraction) (4–5). Desmin, actomyosin, μ -calpain, integrin, and dystrophin are key proteins relevant to the WHC of meat (2,5). However, with thousands of proteins in muscle, these proteins are too few for an understanding of the mechanism of drip loss.

Proteomics is a powerful approach for simultaneous and precise monitoring of proteins, which is helpful for elucidation of molecular connections between muscle proteins and meat quality traits (6–9). Two dimensional electrophoresis (2DE) and MS analysis of porcine exudates have showed that potentially rich sources of protein biomarkers for the WHC (4) include metabolic enzymes, stress response factors, and structural proteins, such as the myosin light chain 1, troponin T, actin (7), the myosin regulatory light chain, α - β -tropomyosin (8), phosphotriose isomerase (4), phosphocreatine

kinase, desmin, and transcription factors (9). Functions of these potential markers for the WHC and drip loss are not fully understood. Furthermore, most studies have focused on pork or chicken. Few studies have been carried out using goat meat. The objectives of this study were (1) identification of differentially expressed proteins in *Longissimus dorsi* (LD) muscles of goats with different drip loss values using 2DE-MS; (2) analysis of relationships between differentially expressed proteins and meat quality traits.

Materials and Methods

Animals and treatments Forty five male goats consisting of 15 Huang-Huai goats, 15 Boer goats, and 15 Boer goat $\times$ Laoshan white goats, aged 9–12 months were conventionally Halal slaughtered by severing the trachea, carotid arteries, and esophagus at a commercial slaughterhouse (Kaiyin Ltd., Dongying, China) in April of 2012. After carcasses were chilled at 4°C for 24 h, the LD muscle tissue between the 1st and 2nd lumbar vertebrae on the left side of the carcass was dissected and sliced into 2 parts. One part was quickly cut into small pieces by a knife (XRD-511; Xinrongda Ltd., Chongqing, China), mixed by a mincer (TC121; Henglian Ltd., Guangzhou, China), snap-frozen in liquid nitrogen, and stored in an ultra low temperature freezer (8926; Thermo Fisher Scientific Inc., Waltham, MA, USA) at –80°C prior to analysis. The other part was used for drip loss measurements. Drip loss was evaluated as previously reported (10). The part of each LD

muscle between the 1st and 2nd lumbar vertebrae was cut by a knife (XRD-511; Xinrongda Ltd.), weighed (W_1) by electronic balance (ML204/02; Mettler Toledo, Columbus, OH, USA) and suspended in a vacuum plastic bag (9.3 mL O₂/m²/24 h, 0, 0.19 mm thick, Magic Seal[®], Dongguan, China) and hung the bag on a rope for 24 h at 4°C. After hanging, the muscle weight was measured by electronic balance (ML204/02; Mettler Toledo) as W_2 , and the drip loss was calculated as drip loss (%) = $(W_1 - W_2)/W_1 \times 100$. Three carcasses with the highest drip loss values within each goat breed were selected and assigned to a high drip loss group, while 3 carcasses with the lowest drip loss values were assigned to a low drip loss group.

Meat quality measurement The LD muscle between lumbar vertebrae 3-4 on the left side of the carcass was used for instrumental measurement of meat quality. The pH was measured via insertion of a pH probe (HI 9916; Hanna Instrument, Padova, Italy) into the LD muscle between the 3rd and 4th lumbar vertebrae using a portable needle-tipped combination electrode (Hanna Instrument) at approximately 45 min postmortem, and the pH value was expressed as pH_{45 min}. After carcasses were chilled in the chilling room for 24 h at 4°C, LD muscles were dissected and kept at 25°C for 30 min, then CIE L^* , a^* , and b^* coordinates (lightness, redness, and yellowness, respectively) were determined using a CR300 Chromameter (Minolta, Osaka, Japan) on a freshly cut surface following the Addis method (11) with a standard white plate as a blank (L_0 , a_0 , b_0). Color differences as ΔE (ΔE) values were calculated as: $\Delta E = [(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2]^{1/2}$.

The LD muscle between lumbar vertebrae 3-4 on the right side of the carcass was used for texture profile of lamb quality following the method described by Wheeler *et al.* (12). The muscles were cooked in hot water at 80°C until the internal temperature reaching 70°C, and then cut with a knife (XRD-511; Xinrongda Ltd.) to 2.54 cm thick as samples. The texture profile of the cooked sample was measured using a TA-XT2i texture analyzer (Stable Micro Systems Ltd., Godalming, UK) with a HDP/BSW probe (Stable Micro Systems Ltd.). The sarcomere length 24 h postmortem was determined using a Helium-Neon laser diffraction technique and an H-7500 transmission electron microscope (Hitachi, Ltd., Tokyo, Japan) following the method described by Palka and Daun (13).

2D proteome analysis Frozen LD muscles were used for gel-based proteome analysis. For each goat breed, 3 muscle samples with the highest and lowest drip loss values were ground into powder in liquid nitrogen with a mortar (90 mm, Dacheng, Qingdezhen, China), and mixed together.

Protein samples One g of muscle powder was homogenized in 10 mL of 8 M urea, 2 M thiourea, 40 mM DTT, 4% CHAPS, and 0.5% IPG buffer for 1 min at 5,000 rpm with a polytron homogenizer (T10 basic; IKA[®] Werke GmbH & Co. KG, Staufen, Germany). Crude extracts were transferred to an Eppendorf tube, vigorously shaken

on the shaking table (TS-2; Qilinbeier Ltd., Haimen, China) for 2 h at 0°C, and centrifuged with a centrifuge (Neofuge 15R; Lishen Ltd., Shanghai, China) at 10,000 $\times g$ for 60 min. Supernatants were collected and protein concentrations were adjusted with buffer solution containing 8 M urea, 2 M thiourea, 40 mM DTT, 4% CHAPS, and 0.5% IPG buffer to 1 mg/mL.

2D electrophoresis Five hundred μ L of a protein extract solution was loaded onto Immobiline Dry Strips (pH 4-7, NL, 24 cm) (GE). After 14 h of passive hydration, strips were fixed on the electrodes of an Ettan IPGphor3 (GE) with isoelectric focusing for more than 11,000 Vh (voltage $\times$ h) at 20 with an electric current no greater than 50 μ A. After balancing in 1% DTT buffer (6 M urea, 75 mM Tris-HCl, 29.3% glycerol, 2% SDS) for 15 min, then in 2.5% iodoacetamide buffer (6 M urea, 75 mM Tris-HCl, 29.3% glycerol, 2% SDS) for 15 min, each strip was put on the top of 12.5% Multiphor II separation gel (GE). The gel was sealed with 1.5 mL low melting point agarose, electrophoresized with 8 w for each gel at a maximum voltage 600 V for 6 h, and then stained with Coomassie Brilliant Blue-G250. Triplicate stained gels for each muscle sample were scanned and protein spots with a 2x difference in density were identified using Image Master 7.02DE image analysis software (GE). Targeted protein spots were excised using a circular plug of 1.5 mm in diameter and subjected to in-gel trypsin digestion.

LC/MS/MS Protein identification was carried out based on LC-MS/MS analysis. A Thermo Scientific LTQ ORBITRAP X L mass spectrometer was connected to an Eksigent NANO LC. 1DPLUS chromatography system equipped with an auto-sampler. Tryptic peptides were re-suspended in 1 mL of 0.1% formic acid. Each muscle sample was loaded onto a Biobasic C18 Picofrit TM column (100 mm length, 75 mm id) and separated using a 25 min reverse phase increasing acetonitrile gradient from 0 to 50% for 11 min at a flow rate of 30 nL/min. The MS apparatus was operated in positive ion mode with a capillary temperature of 200, a capillary voltage of 9 V, a tube lens voltage of 100 V, and with a potential of 1,800 V applied to a frit.

Data were processed using a Mass Lynx Windows NT PC system. Peptide mass values from LC/MS/MS were matched with theoretical peptide values of proteins in the National Center for Biotechnology Information (NCBI) database using MASCOT and/or Profound software. All MS/MS spectra recorded for tryptic peptides were searched against protein sequences from NCBI and Expressed Sequence Tags (EST) databases using the MASCOT search program (7-9).

Statistical analysis Changes in drip loss, pH_{45 min}, L^* , a^* , b^* , E , shear force, and sarcomere length in goat LD muscles were analyzed using General Linear Model (GLM) procedures of the SAS 8.2 package (SAS Institute Inc., Cary, NC, USA). The 2 treatment groups of high drip loss and low drip loss were fixed effects, and muscles were designated as a random effect. Significant differences at $p < 0.05$ were identified

based on Turkey's test of means using the LSMEANS option within the GLM procedure. Mean and standard deviation (SD) values were reported.

Results and Discussion

Meat quality traits Drip loss is an important indicator of meat quality. Low drip loss indicates a high WHC value, greater juiciness, freshness, and a less-dry surface. The $\text{pH}_{45 \text{ min}}$ value, the pH of muscle at 45 min after slaughter, is related to drip loss (14). The $\text{pH}_{45 \text{ min}}$ value of the low drip loss group was significantly ($p<0.05$) higher than for high drip loss group, but there were no significant ($p>0.05$) differences among the 3 breeds within each group (Table 1). The L^* value of the high drip loss group was significantly ($p<0.05$) higher than for the low drip loss group for all breeds, due to more water flowing onto the muscle surface and increasing light reflection. The a^* and b^* values were not significantly ($p>0.05$) different between the 2 groups.

Delta E (ΔE) is defined as the difference between 2 colors in L^* , a^* , and b^* color space. E values were significantly ($p<0.05$) higher in the high drip loss group than in the low drip loss group only for Boer goats. In this study, the Warner Bratzler shear force of the high drip loss group was not significantly ($p>0.05$) different from the low drip loss group, perhaps due to water loss making the LD muscle tougher.

The sarcomere length of the high drip loss group was significantly ($p<0.05$) shorter than for the low drip loss group. With an increase in drip loss, the sarcomere length decreased, due to greater muscle contraction. Sarcomeres were significantly ($p<0.05$) shorter in the high drip loss group than in the low drip loss group.

Differential proteomics analysis The 2 dimensional electrophoresis profiles of goat LD muscles 24 h postmortem are shown in Fig. 1. In total, 158 distinct protein spots were detected. These distinct spots were present at pI values of 4-7 with Mw values from 15 to 200 kDa. After densitometric analysis, 39 protein spots were selected as 15 spots from Huang-Huai goats, 10 spots from Boer goats, and 14 spots from BoerxLaoshan goats, and 22 proteins were identified using LC/MS/MS from these protein spots. The 22 proteins were different in abundance between muscle tissues of high and low drip loss, with most proteins under-expressed in the high drip loss group.

Proteins with significantly ($p<0.05$) different abundance values between the 2 drip loss groups are listed in Table 2, 3, and 4. Compared with the low drip loss group, all differential proteins were remarkably under-expressed in high drip loss muscles of Huang-Huai goats (Table 2). For Boer goats, the fatty acid-binding protein, actin, and ATP synthase subunit beta were over-expressed in high drip loss muscles, while all the other 7 proteins were under-expressed (Table 3). Only the myosin light chain 3 was up-regulated in the high drip

Table 1. Meat quality traits of goat LD muscles with different drip loss values

		High drip loss	Low drip loss
Drip loss/%	Huang-Huai goat	2.27±0.31 ^{a1)}	0.33±0.02 ^b
	Boer goat	2.20±0.16 ^a	0.82±0.16 ^b
	BoerxLaoshan white goat	4.68±0.71 ^a	0.36±0.09 ^b
$\text{pH}_{45 \text{ min}}$	Huang-Huai goat	6.10±0.07 ^b	6.35±0.04 ^a
	Boer goat	6.19±0.05 ^b	6.34±0.08 ^a
	BoerxLaoshan white goat	6.11±0.04 ^b	6.22±0.06 ^a
L^*	Huang-Huai goat	55.89±3.83 ^a	52.47±2.76 ^b
	Boer goat	54.07±1.95 ^a	45.33±0.65 ^b
	BoerxLaoshan white goat	49.82±1.98 ^a	40.86±1.15 ^b
a^*	Huang-Huai goat	10.35±0.20	13.29±1.52
	Boer goat	10.90±0.35	11.20±0.40
	BoerxLaoshan white goat	9.47±0.67	12.55±1.41
b^*	Huang-Huai goat	11.84±0.30	13.35±2.07
	Boer goat	11.64±0.35	13.15±0.47
	BoerxLaoshan white goat	10.91±0.70	11.16±1.26
ΔE	Huang-Huai goat	54.20±1.29	53.13±1.22
	Boer goat	53.01±1.56 ^a	44.04±0.67 ^b
	BoerxLaoshan white goat	50.52±1.77	47.68±2.04
Shear force/Kg	Huang-Huai goat	7.18±0.60	6.66±1.49
	Boer goat	7.47±0.50	7.06±0.67
	BoerxLaoshan white goat	7.43±1.07	7.01±1.26
Sarcomere length/ μm	Huang-Huai goat	1.27±0.33	1.38±0.31
	Boer goat	1.26±0.15	1.36±0.11
	BoerxLaoshan white goat	1.37±0.26 ^b	1.97±0.30 ^a

¹⁾Data are reported as mean±SD ($n=3$). Different superscripts in the same row indicate significant differences ($p<0.05$).

Fig. 1. 2DE electrophoresis profiles of goat LD muscle tissues with different drip loss values. A_{LD}- Longissimus dorsi of Huang-Huai goats with low drip loss, A_{HD}- Longissimus dorsi of Huang-Huai goats with high drip loss, B_{LD}- Longissimus dorsi of Boer goats with low drip loss, B_{HD}- Longissimus dorsi of Boer goats with high drip loss, C_{LD}- Longissimus dorsi of Boer×Laoshan white goats with low drip loss, C_{HD}- Longissimus dorsi of Boer×Laoshan white goats with high drip loss.

loss group and 13 proteins were down-regulated in Boer × Laoshan white goats (Table 4). For the 22 differentially expressed proteins, higher frequencies among the 3 goat breeds were identified for α -enolase X2 isomers (α -enolase isoform X2), cytochrome b-c1 complex subunit 1 (cytochrome b-c1 complex subunit 1), the β subunit of pyruvate dehydrogenase (pyruvate dehydrogenase E1 component subunit beta), the myosin light chain (myosin light chain), superoxide dismutase (superoxide dismutase), myosin (myosin), peroxide redox enzymes (peroxiredoxin-2), heat shock protein 27 (HSP27 protein), and NADH dehydrogenase (NADH dehydrogenase). These proteins are the metabolic enzymes enolase, NADH dehydrogenase, and pyruvate dehydrogenase, the stress response factors HSP27, peroxiredoxin, and superoxide dismutase, and the structural proteins myosin and the myosin light chain, that affect glycolysis, oxidation, and muscle contraction. The myosin light chain, HSP27, the cytochrome b-c1 complex subunit 1, superoxide dismutase,

myosin, and actin were identified in more than a single spot in the same gel, suggesting that they may represent multiple isoforms, fragments, cross links, or post-translational modification (PTM). Differential proteins related to glycolysis, oxidation, and muscle contraction will be verified using either Western blotting or mRNA expression in future studies.

Metabolic enzymes and drip loss After slaughter, glycogen and glucose in muscles break into lactic acid via glycolysis, lowering the pH of muscle tissue from approximately 7.0 to 5.3-5.8 (15). The decline of pH causes some proteins reach the isoelectric point (pI), which results in much lower WHC values for meat (15). The glycolytic enzymes calpain and cathepsin are crucial to meat water holding capacity values (16). α -Enolase is a glycolytic enzyme. After exsanguination, the oxygen supply to muscle tissue is cut off and glycolysis ensues. Enolase is responsible for catalysis of the conversion of 2-

Table 2. Identification of proteins different in abundance between low and high drip loss LD muscle tissues from Huang-Huai goats using LC-MS/MS

Spot No. ¹⁾	Protein ²⁾	Accession no.	Source	Theoretical PI/MW ³⁾	Matched peptides ⁴⁾	Sequence coverage	H vs. L ⁵⁾
1	myosin light chain 6B	gi 548466316	Capra hircus	5.40/23488	443(374)	68%	-
2	myosin light chain 6B	gi 548466316	Capra hircus	5.40/23488	443(374)	68%	-
3	NADH dehydrogenase [ubiquinone] iron-sulfur protein	gi 548496668	Capra hircus	5.64/26687	129(100)	55%	-
4	HSP27 protein	gi 389620461	Capra hircus	6.21/20543	318(199)	59%	-
5	C->U-editing enzyme APOBEC-2	gi 548517020	Capra hircus	4.84/26027	118(81)	72%	-
6	pyruvate dehydrogenase E1 component subunit beta	gi 548515230	Capra hircus	6.21/39485	246(186)	54%	-
7	alpha-enolase isoform X2	gi 548498577	Capra hircus	5.92/49376	309(221)	59%	-
8	thioredoxin-dependent peroxide reductase	gi 548523749	Capra hircus	7.75/28377	459(399)	33%	-
9	cytochrome b-c1 complex subunit 1	gi 548515658	Capra hircus	5.84/51307	165(99)	31%	-
10	HSP27 protein	gi 389620461	Capra hircus	6.21/20543	64(32)	45%	-
11	retinoblastoma-associated protein	gi 548487941	Capra hircus	8.90/94636	10(7)	1%	-
12	eukaryotic translation initiation factor 5A-1	gi 548507861	Capra hircus	5.08/17049	275(172)	66%	-
13	peroxiredoxin-2	gi 548472190	Capra hircus	5.56/20157	10(7)	66%	-
14	cytochrome b-c1 complex subunit 1	gi 548515658	Capra hircus	5.84/51307	342(239)	55%	-
15	succinyl-CoA ligase [ADP-forming] subunit beta	gi 548487933	Capra hircus	5.52/43878	426(337)	66%	-

¹⁾Spot numbers refer to proteins labeled in A_{LD} and A_{HD} in Fig. 1.

²⁾Protein names in the NCBI database.

³⁾Theoretical isoelectric point (pI) and Mw were obtained from NCBI.

⁴⁾Percentage of amino acids in reference proteins covered by matching peptides obtained using MS.

⁵⁾H vs. L means high drip loss is compared to low drip loss. "+" and "-" represent over-expressed and under-expressed, respectively.

Table 3. Identification of proteins different in abundance between low and high drip loss LD muscle tissues from Boer goats using LC-MS/MS

Spot No. ¹⁾	Protein ²⁾	Accession no.	Source	Theoretical PI/MW ³⁾	Matched peptides ⁴⁾	Sequence coverage	H vs. L ⁵⁾
1	superoxide dismutase	gi 58865328	Capra hircus	5.85/15871	194(174)	39%	-
2	fatty acid-binding protein	gi 39939390	Capra hircus	6.11/14810	201(146)	68%	+
3	superoxide dismutase	gi 58865328	Capra hircus	5.85/15871	46(23)	25%	-
4	pyruvate dehydrogenase E1 component subunit beta	gi 548515230	Capra hircus	6.21/39485	343(273)	54%	-
5	myosin-1	gi 548508120	Capra hircus	5.55/224715	201(118)	13%	-
6	cytochrome b-c1 complex subunit 1	gi 548515658	Capra hircus	5.84/51307	461(379)	58%	-
7	actin	gi 548482267	Capra hircus	5.30/42437	247(177)	59%	+
8	alpha-enolase isoform X2	gi 548498577	Capra hircus	5.92/49376	370(262)	65%	-
9	ATP synthase subunit beta	gi 548466133	Capra hircus	5.14/56148	425(332)	67%	+
10	myosin-2	gi 548508115	Capra hircus	5.59/222489	311(195)	10%	-

¹⁾Spot numbers refer to proteins labeled in B_{LD} and B_{HD} in Fig. 1.

²⁾Protein names from the NCBI database.

³⁾Theoretical isoelectric point (pI) and Mw values were obtained from NCBI.

⁴⁾Percentage of amino acids in reference proteins covered by matching peptides from MS.

⁵⁾H vs. L means high drip loss is compared to low drip loss. "+" and "-" represent over-expressed and under-expressed, respectively.

phosphoglycerate to phosphoenolpyruvate (17). The reaction is reversible as a phosphopyruvate hydratase, which catalyzes phosphoenolpyruvate to 2-phosphoglycerate (17). In addition, enolase is involved in transcription, development, and apoptosis (18). More enolase might induce phosphoenolpyruvate conversion to 2-phosphoglycerate, and prevention of cellular apoptosis and myofibrillar breakage that result in higher WHC and lower drip loss values.

NADH dehydrogenase located in the mitochondrial inner membrane for catalysis of electron transmission from NADH to coenzyme Q is the beginning of mitochondrial oxidative phosphorylation, which is the principle pathway for synthesis of ATP in anaerobic metabolism

in which electrons are delivered from NADH or FADH₂. Down-regulated expression of NADH dehydrogenase in high drip loss muscle tissues results in lower activity and faster termination of electron transfer, leading to a decrease in ATP production for late biochemical reactions. ATP was consumed more quickly in the high drip loss group, so more anaerobic respiration was conducted to generate more lactic acid, and pH values declined.

Pyruvate dehydrogenase E1 is a component of the pyruvate dehydrogenase complex that catalyzes irreversible oxidative decarboxylation of pyruvic acid to form acetyl-CoA for entrance into the tricarboxylic acid cycle. Down-regulated pyruvate dehydrogenase E1 in the high drip loss muscle group might prevent formation of

Table 4. Identification of proteins different in abundance between low and high drip loss LD muscle tissues from Boer×Laoshan goats using LC-MS/MS

Spot No. ¹⁾	Protein ²⁾	Accession no.	Source	Theoretical PI/MW ³⁾	Matched peptides ⁴⁾	Sequence coverage	H vs. L ⁵⁾
1	myosin light chain 3	gi 548515867	Capra hircus	4.99/22088	213(145)	76%	+
2	peroxiredoxin 2	gi 379067372	Capra hircus	5.37/22203	134(110)	50%	-
3	apolipoprotein A-I-like	gi 548495112	Capra hircus	5.39/23622	438(293)	70%	-
4	superoxide dismutase	gi 58865328	Capra hircus	5.85/15871	194(174)	39%	-
5	pyruvate dehydrogenase E1 component subunit beta	gi 548515230	Capra hircus	6.21/39485	343(273)	54%	-
6	actin	gi 548524919	Capra hircus	5.31/42249	318(225)	30%	-
7	myosin-1	gi 548508120	Capra hircus	5.55/224715	201(118)	13%	-
8	actin, alpha cardiac muscle 1 isoform X1	gi 548482267	Capra hircus	5.23/42381	360(249)	64%	-
9	cytochrome b-c1 complex subunit 1	gi 548515658	Capra hircus	5.84/51307	268(210)	50%	-
10	actin	gi 548482267	Capra hircus	5.30/42437	247(177)	59%	-
11	tripartite motif-containing protein 72	gi 548521853	Capra hircus	6.34/29647	149(84)	49%	-
12	alpha-enolase isoform X2	gi 548498577	Capra hircus	5.92/49376	370(262)	65%	-
13	cytochrome b-c1 complex subunit 1	gi 548515658	Capra hircus	5.84/51307	265(222)	50%	-
14	triosephosphate isomerase	gi 548468261	Capra hircus	6.12/25538	240(189)	56%	-

¹⁾Spot numbers refer to proteins labeled in C_{LD} and C_{HD} in Fig. 1.

²⁾Protein name from the NCBI database.

³⁾Theoretical isoelectric point (pI) and Mw values were obtained from NCBI.

⁴⁾Percentage of amino acids in reference proteins covered by matching peptides from MS.

⁵⁾H vs. L means high drip loss compared with low drip loss. "+" and "-" represent over-expressed and under-expressed, respectively.

acetyl-CoA and prevent aerobic respiration, and might promote production of lactic acid even when oxygen is still available. Declines in α -enolase, NADH dehydrogenase, and pyruvate dehydrogenase levels may promote glycolysis and accumulation of lactic acid in muscle tissues, leading to lower pH and higher drip loss values.

Stress response factors, protein function, and drip loss Stress response factors stabilize functional conformation of proteins, regulate stability and activity of enzymes, protect cells under stress, and reorient broken proteins in proteolysis. Moreover, stress response factors play a key role in anti-oxidation and anti-apoptosis (19,20). Heat shock protein HSP27, peroxiredoxin, and superoxide dismutase were the main stress response factors related to drip loss of goat meat in this study. HSP27 plays a key role in maintaining the integrity of the myofibril structure, especially the integrity of the Z line and the I band (20). Guay *et al.* (21) reported that down-regulated expression of HSP27 caused actin polymerization and actin microfilament stabilization, while lower HSP27 levels accelerated actin denaturation (22). Kim *et al.* (23) reported that HSP27 was positively correlated with shear force and indicated that down-regulated expression of HSP27 might lead to rapid degradation of muscle proteins (23), all in agreement with this study that the quantity of HSP27 was higher in the low drip loss group than in high drip loss group, and that shear force showed opposite results. Besides, HSP27 can be phosphorylated to prevent degradation in oxidative stress or in interactions with actin (22,24). Although the role of HSP27 is not fully understood in meat WHC values, Laville *et al.* (25) reported that a form of HSP27 was absent from pale, soft and exudative (PSE) pork when compared with normal pork. Yu *et al.* (26)

reported that increased drip loss in pork from pre-slaughter stressed pigs was related to decreased expression of HSP27 in muscle tissues. The HSP27 level decreased when the pH decreased in this study, causing myofibril structural damage, leading to an increase in drip loss.

As a free radical scavenger, superoxide dismutase (SOD) is a key anti-oxidant enzyme that is involved in anti-aging and prevention of biomolecular damage (27). Free radical levels increase when the SOD activity decreases within the body, resulting in induction of oxidation and damage to muscle fibers. Peroxiredoxin-2 (PRDX2) is an antioxidant protein located in mitochondria that inhibits lipid oxidation and regulates the phospholipid metabolism via scavenging peroxide in cells and, thus, plays an important role in electron transfer and cellular oxidation defense (28).

Studies showed that oxidation is a dominant factor causing meat quality deterioration. Generally, oxidation decreases the fluidity of the cell membrane and breaks the normal fiber structure. Consequently, muscle cell integrity is destroyed and cell fluid flows out, leading to high drip loss and an inferior meat quality (29). Previous studies have reported that lipid oxidation is positively correlated with drip loss (30). Expression of SOD and PRSX2 is down-regulated in PSE muscle, which implies that low SOD and PRDX2 contents weaken the anti-oxidation activity of muscle, resulting in an increase in lipid oxidation. High lipid oxidation levels would damage the membrane structure and increase the amount of drip loss (30).

Structural proteins and the water holding capacity of meat Myosin is the most abundant protein in thick myofibrillar muscle tissues. A myosin molecule is composed of 2 myosin heavy chains weighting

220 kDa and 2 myosin light chains weighting 16 - 27 kDa. Myosin has an ATPase activity for hydrolysis of ATP for contraction. Phosphorylation of myosin increases the ATPase activity and muscle contraction, while de-phosphorylation of myosin induces muscle relaxation (31). Abnormal muscle contraction and relaxation postmortem both increase drip loss (4-5). Myosin is the principle protein responsible for water retention. When the muscle tissue pH declines to the pI value of myosin, the distance between thick and thin myofilaments is reduced to <2.5 nm and the sarcomere length shortens (32), allowing water in the myofibril gap to flow out, in agreement with this study where the sarcomere length was shorter in the high drip loss group. Meanwhile, the myosin head was degenerated and unable to stabilize more water, resulting in more drip loss.

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