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Research Article

Differential Expression of Proteins in Datong Yak and Chaidamu Yellow Cattle *Longissimus lumborum* Muscles and Relation to Meat Water Holding Capacity

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Abstract

We investigated that proteins differently expressed in Datong Yak and Chaidamu Yellow Cattle *Longissimus longurum* muscles and their relation to tissue water-holding capacity. Samples were classified according to breed and postmortem aging into Yak0h, Cattle0h, Yak24h and Cattle24h groups. Fifty seven differentially expressed proteins were confirmed by MALDI-TOF/TOF-MS. Twenty eight proteins could be identified and were divided into five main categories: structural proteins, metabolic enzymes, stress related proteins, transporter proteins and binding proteins. Myosin light chain (MLC), Heat Shock 27kDa (HSP 27) and Keratin 10 (KRT 10) proteins showed significant differences in expression between yak and cattle meat and may have the potential to be used as biological markers of tissue WHC. Bioinformatics analysis showed differentially these proteins included both metabolic enzymes and structural proteins. The functions of the identified proteins contribute to a more detailed molecular view of the processes behind WHC and are a valuable resource for future investigations.

Keywords: *Bos taurus, LL muscles,* Water-holding capacity, *Meat quality, Proteomics, Bioinformatics*

Datong Yak ve Chaidamu Sarı Sığırı *Longissimus lumborum* Kasında Proteinlerin Farklı Ekspresyonu ve Etin Su Tutma Kapasitesi İle İlişkisi

Öz

Bu çalışmada Datong Yak ve Chaidamu Sarı Sığırı *Longissimus lumborum* kasında proteinlerin farklı ekspresyonu ve bu proteinlerin etin su tutma kapasitesi ile ilişkisi araştırılmıştır. Örnekler ırka ve postmortem yaşlanmaya göre sınıflandırıldı (Yak0h, Cattle0h, Yak24h ve Cattle24h). Elli yedi farklı eksprese edilen protein MALDI-TOF/TOF-MS ile onaylandı. Yirmi sekiz protein tanımlanarak yapısal proteinler, metabolik enzimler, stres ilişkili proteinler, taşıyıcı proteinler ve bağlayıcı proteinler olmak üzere beş ana kategoriye ayrıldı. Myosin hafif zincir, Isı şok 27kDa (HSP 27) ve Keratin 10 proteinlerinin yak ve sığır etleri arasında ekspresyon bakımından önemli farklar göstermesi sebebiyle bu proteinlerin doku su tutma kapasitesi için biyolojik marker olarak kullanılabilecekleri kanısına varıldı. Biyoinformatik analizi bu proteinlerin farklı metabolik enzimler ve yapısal proteinleri içerdiğini gösterdi. Tespit edilen proteinlerin fonksiyonları su tutma kapasitesinin arkasında yatan daha derinlemesine moleküler ilişkiye ışık tutmakta ve gelecek araştırmalar için değerli bir kaynak oluşturmaktadır.

Anahtar sözcükler: *Bos taurus, LL kası, Su tutma kapasitesi, Et kalitesi, Proteomiks, Biyoinformatik*

INTRODUCTION

Yak *(Bos grunniens)* mainly live above an altitude of 3000 meters in the Tibetan plateau. The yak meat has a low fat content, however, the commercial potential of yak meat is limited, in part due to a lack of data regarding meat quality [1]. The physical traits of meat (e.g., tenderness, water holding capacity (WHC) and chromatic features) are widely used for the evaluation of red meat quality during processing and storage [2]. Muscle pH and protein denaturation are used as the main determinants of WHC, informing on meat quality [3]. The rapid degradation of proteins lead to the net charges of the muscle proteins being reduced, which forces more water out of the meat. In addition to protein denaturation, this influences its appearance, palatability and processability [4].

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The degree of protein denaturation, particularly cytoskeletal proteins in carcass meat, often depends on its WHC and pH [5]. High degrees of myofibrillar and sarcoplasmic protein denaturation could promote myofibril shrinkage and force water out. Some reports show that meat with low WHC and low pH was caused by cellular water loss from the myofibrillar matrix [6,7]. However, others consider that WHC may also increase with ageing due to the breakdown in meat structure and the creation of a "sponge effect", which disrupts the channels through which moisture is lost, physically entrapping free water in meat [8]. Changes in meat during postmortem aging are suggested to be highly coordinated, genetically programmed and an irreversible phenomenon involving a series of physiological, biochemical, and proteomic changes [9]. These varied reports make WHC a promising target for continued studies of meat quality mechanism, though at present little is known about the relationship between WHC and protein changes.

Proteomics has been widely used to predict the meat quality [10]. Numerous two-dimensional electrophoresis (2-DE) gel based studies have already reported the relationships between factors such as tenderness, color, pre-slaughter stress and WHC [11-14]. However, while WHC characteristics play an important role in the dynamic nature of meat postmortem muscle qualities, the biological mechanisms involved in the structural and biochemical changes are not fully understood during the ageing process.

In the present study, we wanted to confirm the relationships between changes in physical meat properties (pH, color, water holding capacity) and related protein changes in the *Longissimus lumborum* (LL) muscle both in Datong Yak and Chaidamu Yellow Cattle as determined by proteomic and bioinformatic methods. Our research objectives were therefore to screen the differential expression of proteins in yak and cattle muscles related to the WHC of meat and to explore the potential molecular mechanisms underlying the proteomic data.

MATERIAL and METHODS

The study protocol was approved by the Regulations for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China, revised in 2004) and approved by the Institutional Animal Care and Use Committee (State key of laboratory of Plateau Ecology and Agriculture, Qinghai University, China, 2015).

Animals and Sample Preparation

Five Datong yak bulls and five Chaidamu yellow cattle bulls of the same age (4±0.5 years old) were stunned by captive bolt pistol and their blood was drained. Animal slaughtering was approved by the National Administration of Cattle Slaughtering and Quarantine regulations (Qinghai, China). The Datong Yak and Chaidamu Yellow Cattle were randomly choosen from experimental base of Qinghai University (Lvcaoyuan Food Ltd., Datong City, Qinghai Province, China) and the LL muscles were excised from the left half of each carcass at the 11th rib. *M. longissimus thoracis* (100 g) from each carcass was divided into two parts, the first one was analyzed at 0 h after slaughter in 18°C; the second one was aged for 24 h in a refrigerated chamber at 4°C and relative humidity of 98%. Any blood or other contaminants on the LL muscle surface were removed by swabbing with phosphate buffered saline solution, then frozen in liquid nitrogen, and stored at -80°C until the extraction of muscle proteins.

Measurement of Meat Qualities

Intramuscular fat content was analyzed using methods of the Association of Official Analytical Chemists [15]. pH was measured using a portable pH meter (SenvenGo, Mettler-Toledo, Switzerland) at 45 min (pH_{0h}) and 24 h (pH_{24h}) [16] after slaughter. The electrode was first calibrated at a temperature of 22°C which and used a two-point calibration, and the pH of the calibration buffer used was 7.000 and 4.005 at 25°C.

ACR-400 Minolta colorimeter (Konica Minolta Sensing Americas Inc., Ramsey, NJ, USA) was applied to determine the lightness (L*), redness (a*) and yellowness (b*) of the meat samples at 0 h (exposed to air directly for 30 min at 18°C). For each parameter, values were measured on 5 sites of each sample, respectively [17].

Water holding capacity (WHC) was determined following compression by the filter paper press method [4]. To measure the strength of the meat tissue, Warner-Bratzler (WB) shear force was measured on cooked meat (2.54 cm thick) per muscle according to the protocol of Wheeler [18]. A transversal section of the LL muscle for each animal was cooked to a core temperature of 70°C in a pre-heated water bath, subsequently cooled in running water for 30 min to reach a core temperature below 30°C. The cores (1.27 cm, parallel to longitudinal orientation of fibres) were then taken from each sample and peak force was determined using a V-shaped shear blade with a cross-head speed of 400 mm/min.

Extraction of Muscle Proteins

According to Jia's Method [19], frozen muscle tissue (30 mg) was placed in a mortar and ground in liquid nitrogen into a powder. Then, 0.5 g muscle tissue sample was added to 1 mL lysis buffer. Samples were lysed at room temperature for 30 min followed by homogenization with 22% vibrating amplitude via ultrasonic amplitude in ice water (pulsed ultrasound was programmed to turn on for 10 s and then off for 15 s, for a total of 250 s). Samples were placed at 4°C for 4 h and then centrifuged at 14.000 rpm at 4°C for 30 min. The supernatant was retained and protein solution was exchanged into 50 mmol/L triethy1-

ammonium bicarbonate, pH 9, using a PD10 column according to manufacturer instructions (GE Healthcare). Total protein content was measured using the Bradford method (IMPLEN, NanoPhotometerTM), and the lysates stored at -80°C.

Two-dimensional Electrophoresis (2-DE) and Mass Spectrometry Analysis

For 2-DE, the muscle protein extract samples approximately 200 µg were performed to 17 cm nonlinear immobilized pH gradient (IPG) strips (pH 3-10, Bio-Rad). After hydration of the IPG strips at 50 V for 14 h, isoelectric focusing (IEF) was performed as follows: 1 h at 500 V, 1 h at 1000 V to remove salts, followed by 6 h at 1000-9000 V by a linear voltage for 80 000 Vh, and finally 500 V for the hold period. PROTEAN® IEF Cell PROTEAN i-12 (Bio-Rad) was selected for IEF, and the current limit was adjusted to 50 mA per strip; the run was carried out at 20°C. An 12.5% SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) was used for secondary separation using a Protean II XL system (Bio-Rad). The SDS-PAGE steps were as follows: 50 V for 1 h and 150 V for 9 h. Gels were stained with Coomassie blue G-250 [20].

Five biological repeats were performed for each treatment. Every independent biological replicate 2DE gel image was captured using a GS-900® Calibrated Densitometer (Bio-Rad) at grayscale of 256 units and resolution of 600 dpi. PDQuest 8.0.1 software was used to perform the image filtration, background subtraction, spot detection, spot matching, and quantitative intensity analyses. Matched spots exhibiting a statistically significant ($P<0.05$) 2 fold or more intensity difference were considered as differentially abundant.

Matched spots were manually excised by micro pipette tips from the gels. The samples were analysis by mass-spectrophotometry by APT Co., LTD (Shanghai, China). Tryptic peptide analysis was performed using a 4800 Plus MALDI TOF/TOF Analyzer (Applied Biosystems, USA). The mass spectrometry (MS) was performed as follows: enzymolysis, ziptip-desalination, peptide extraction in enzyme solution, MALDI-TOF/TOF, data analysis and protein identification [21]. The data of mass and mass/mass spectra were searched through Mascot (Version 2.1, Matrix Science, Boston,MA, USA) and the corresponding proteins were matched against bovine Information (NCBI) database.

Bioinformatics

The differentially expressed proteins which were identified by PDQuest 8.0.1 software and MS were analyzed by Gene ontology (GO) by blast2GO 2.8 software. KEGG showed a pathway enrichment analysis of the differentially expressed proteins, the filter was e-value < $1e^{-10}$ and match score > 65. The PPi are the proteins, and the edges represent the predicted functional associations using Cyposcape 3.0 software.

Statistical Analysis

Statistical significance was assessed with T-test using PROC TTEST or MIXED model (SAS®, Cary, NC, USA). Experiments adopted completely random design. The differences between means were detected using student's T test at 5% significance ($P<0.05$).

RESULTS

Characteristics of Meat Quality in Datong Yak and Chaidamu Yellow Cattle

The results for meat quality traits of Datong Yak (hereon referred to as Yak) and Chaidamu Yellow Cattle (hereafter referred to as cattle) LL muscles are displayed in *Table 1*.

There were significant differences in the pH of the intracellular water of yak and cattle ($P<0.05$) at 45 min (pH 7.06 Vs. pH 6.69), but no significant differences ($P>0.05$) at 24 h (pH 5.73 Vs. 5.61). This result could be related to living environment of yak and cattle and those life habits leading to a lower fibre conduit, and less binding water capacity in yak [22]. There were significant differences ($P<0.05$) between 0 h and 24 h (pH 7.06 Vs. pH 5.73 and pH 6.69 Vs. pH 5.61), respectively. Lactic acid would gradually product and accumulate inside the muscles during the storage, which had caused pH to decrease [23].

Identification of Differentially Expressed Proteins

In present study, 61 spots displayed significant differences in the level of protein expression ($P<0.05$) at 0 h and 24 h or between yak and cattle LL muscles *(Fig. 1)*. Among them, 57 proteins were successfully identified by MALDI-TOF/TOF-MS. 4 spots were not identified because of the poor database search scores (score<65), which were lower than the 95% threshold required to yield unambiguous results. Because some spots identified repeat proteins, only 28 different proteins were successfully named *(Table 2)*. Some spots were identified as identical proteins, which indicates that they may represent multiple isoforms, fragments and cross linked proteins in the gel based analysis. The differently expressed proteins at 0 h and 24 h between yak and cattle were grouped into six functional classes

Table 1. *Changes of quality characteristics in yak and cattle* LL muscle

Meat Traits	Yak	Cattle
pH_{0h}	7.06±0.16**	6.69±0.18
pH_{24h}	5.73±0.12	5.61±0.11
*a** value	23.8±1.65**	17.14±0.61
*b** value	9.03±1.21	9.71±1.89
*L** value	39.26±5.54	43.23±3.98**
WHC (%)	56.22±1.48*	51.87±1.28
WBSF (kg/cm^2)	4.91±0.13**	4.22±0.14
Intramuscular fat (g/100 g)	2.05±0.15	3.07±0.18**

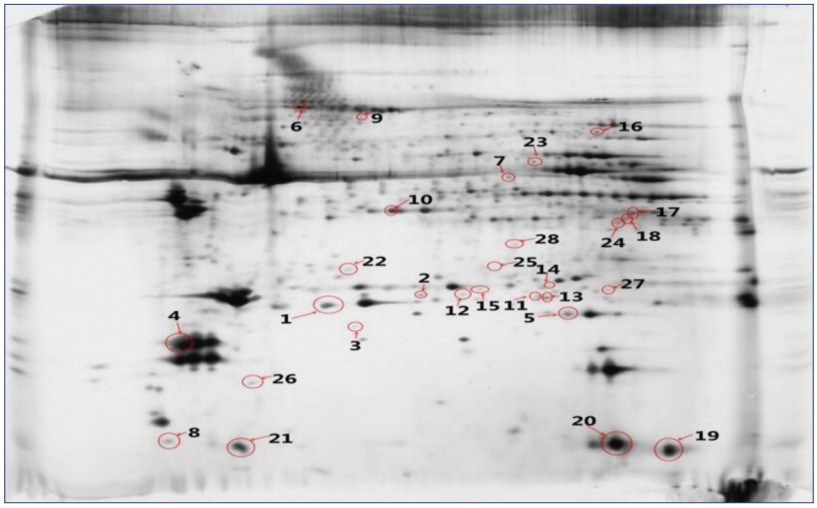


Fig 1. 2DE gel images of proteins extracted from yak and Cattle LL muscle at days 0 and 1 during post-mortem aging. The protein loading was 200 µg and the gel was stained with Coomassie blue 600G-250. 28 spots identified were found to be significantly different in abundance and listed in *Table 2*

Table 2. *Identification of 28 differentially expressed proteins by 2DE and MALDI-TOF/TOF analysis in yak and cattle*

Sample		Protein Name	Accession No.	Species	MW	PI	Score/ Peptides
Structural Proteins	1	Myosin, light chain 6B	tr|Q148H2	*Bos taurus*	23502.3	5.4	44/5
	2	Keratin 10	tr|A6QNZ7	*Bos taurus*	54987.2	5.01	144/15
	3	Actin, alpha	tr|A4IFM8	*Bos taurus*	42338	5.23	412/7
	4	Myosin regulatory light chain 2	sp|Q0P571	*Bos taurus*	19114.4	4.91	199/10
	5	Alpha-crystallin B chain	sp|P02510	*Bos taurus*	20024.4	6.76	308/13
	6	Myosin-7	sp|Q9BE39	*Bos taurus*	223889.2	5.58	414/51
	7	MYH1 protein	tr|Q05B72	*Bos taurus*	34018	5.76	526/24
	8	Keratin 4	tr|A4IFP2	*Bos taurus*	58466.6	7.46	119/16
	9	Myosin-2	tr|F1MRC2	*Bos taurus*	224106.4	5.63	707/44
	10	Troponin T	sp|Q8MKH6	*Bos taurus*	31265.1	5.71	456/11
Glycolytic Enzyme	11	Triosephosphate isomerase	sp|Q5E956	*Bos taurus*	26900.9	6.45	634/14
	12	Thioredoxin-dependent peroxide reductase	sp|P35705	*Bos taurus*	28405.5	7.15	211/9
	13	Adenylate kinase isoenzyme	sp|P00570	*Bos taurus*	21764.3	8.4	467/100
	14	Flavin reductase (NADPH)	sp|P52556	*Bos taurus*	22232.4	6.58	159/4
	15	Triosephosphate isomerase	sp|Q5E956	*Bos taurus*	26900.9	6.45	695/17
	16	ATP synthase subunit alpha	tr|F1MLB8	*Bos taurus*	59766.7	9.21	358/21
	17	Glyceraldehyde-3-phosphate dehydrogenase	tr|Q712W6	*Bos taurus*	28908	9.21	147/7
Stress Related Proteins	18	Heat shock 27kDa protein	sp|Q5E956	*Bos taurus*	22436.3	5.98	720/14
Transporter Proteins	19	Hemoglobin subunit alpha	sp|P01966	*Bos taurus*	15174.9	8.07	571/12
	20	Hemoglobin beta	tr|D4QBB4	*Bos taurus*	16001.3	7.01	741/12
Binding Proteins	21	Galectin-1	sp|P11116	*Bos taurus*	15076.4	5.37	289/8
	22	Prohibitin	sp|Q3T165	*Bos taurus*	29842.9	5.57	594/9
	23	Elongation factor Tu	sp|P49410	*Bos taurus*	49709.1	6.72	297/16
	24	Myozenin-1	sp|Q8SQ24	*Bos taurus*	31653.8	9.17	256/10
	25	Beta-defensin	tr|G8CY11	*Bos taurus*	15026.3	8.17	42/4
Other Proteins	26	Eukaryotic translation initiation factor	sp|Q6EWQ7	*Bos taurus*	17049.5	5.08	269/5
	27	Es1 protein	r|Q3T0U3	*Bos taurus*	29023.1	8.76	58/8
	28	Serine/arginine-rich-splicing factor 6	tr|F1MXY9	*Bos taurus*	39809.5	11.47	42/10

that included structural proteins, metabolic enzymes, stress related proteins, transporter proteins, binding proteins and other proteins.

There were 11 proteins for which expression was down-regulated at 24 h compared to 0 h in cattle ($P<0.05$, *Table 3*). Beta-defensin, Flavin reductase (NADPH), Hemoglobin

Table 3. *Fold change of cattle 0 h Vs 24 h in differentially expressed proteins*

Spot No.	0 h	24 h	Protein Name
1722	-3.53173	3.53173	Beta-defensin
1853	-1.89029	1.89029	Flavin reductase (NADPH)
2083	1.77176	-1.77176	Hemoglobin subunit alpha
1369	-1.67566	1.67566	MYH1 protein
1879	-1.61470	1.61470	Keratin 10
1601	-1.59417	1.59417	Heat shock 27kDa protein
1718	-1.59081	1.59081	Serine/arginine-rich-splicing factor 6
1974	1.58358	-1.58358	Alpha-crystallin B chain
1878	-1.54175	1.54175	Es1 protein
1891	-1.25692	1.25692	Myosin, light chain 6B
1883	-1.23285	1.23285	Thioredoxin-dependent peroxide reductase

Table 4. *Fold change of yak 0 h Vs 24 h in differentially expressed proteins*

Spot No.	0 h	24 h	Protein Name
1588	3.78994	-3.78994	Triosephosphate isomerase
1667	2.90849	-2.90849	Myosin, light chain 6B
1600	-2.77437	2.77437	Heat shock 27kDa protein
1735	2.56359	-2.56359	Keratin 10
1661	-2.35641	2.35641	Thioredoxin-dependent peroxide reductase
1444	2.14926	-2.14926	Prohibitin
1684	2.12196	-2.12196	Actin, alpha 1
1736	-1.85353	1.85353	Myosin regulatory light chain 2
1394	1.84813	-1.84813	MYH1 protein
1815	1.82861	-1.82861	Eukaryotic translation initiation factor
1905	1.69458	-1.69458	Galectin-1
1689	1.65970	-1.65970	Alpha-crystallin B chain
1650	1.57537	-1.57537	Adenylate kinase isoenzyme
1278	-1.55399	1.55399	Glyceraldehyde-3-phosphate dehydrogenase

subunit alpha, MYH1 protein, Keratin 10, Heat shock 27 kDa protein, Serine/arginine-rich-splicing factor 6, Alpha-crystallin B chain, Es1 protein, Myosin light chain 6B, Thioredoxin-dependent peroxide reductase were upregulated at 24 h and the proteins Hemoglobin subunit alpha and Alpha-crystallin B chain were down-regulated.

There were 14 differently expressed proteins between 0 h and 24 h in yak were down- regulated at 24 h ($P<0.05$, *Table 4)*, including triosephosphate isomerase, myosin light chain 6B, heat shock 27 kDa protein, keratin 10, thioredoxin-dependent peroxide reductase, prohibitin, actin alpha, myosin regulatory light chain 2, MYH1 protein, eukaryotic translation initiation factor, myosin-2, galectin-1, alpha-crystallin B chain, adenylate kinase isoenzyme, glyceraldehyde-3-phosphate dehydrogenase. Whereas heat shock 27 kDa protein, thioredoxin-dependent peroxide reductase, myosin regulatory light chain 2 and glycer-aldehyde-3-phosphate dehydrogenase were up-regulated in expression at 24 h.

Table 5. *Fold change of yak Vs cattle at 0 h in differentially expressed proteins*

Spot No.	Yak	Cattle	Protein Name
1749	2.57159	-2.57159	Triosephosphate isomerase
1851	2.18073	-2.18073	Alpha-crystallin B chain
710	-2.03445	2.03445	Myosin-7
1970	-1.83371	1.83371	Hemoglobin beta
1270	1.78043	-1.78043	MYH1 protein
1812	1.76758	-1.76758	Adenylate kinase isoenzyme
1950	1.70511	-1.70511	Keratin 10
1636	1.54484	-1.54484	Actin, alpha
1450	-1.51909	1.51909	Troponin T
1973	1.50355	-1.50355	KRT4 protein

There were ten proteins for which expression were up-regulated in between yak compared to cattle at 0 h ($P<0.05$, *Table 5)*. Triosephosphate isomerase, Alpha-crystallin B chain, Myosin-7, Hemoglobin beta, MYH1 protein, Adenylate kinase isoenzyme, Keratin 10, Actin alpha, Troponin T, KRT4 protein). And Myosin-7, Hemoglobin beta and Troponin T were down-regulated expression in yak compared to cattle.

There were 13 proteins which were expressed more in cattle LL muscle than in yak at 24 h ($P<0.05$, *Table 6)*, namely keratin 10, myosin-2, troponin T, heat shock 27kDa protein, thioredoxin-dependent peroxide reductase, ATP synthase subunit alpha, KRT4 protein, myosin-7, myosin light chain 6B, elongation factor Tu, MYH1 protein, actin alpha and myozenin-1. Myosin-2, heat shock 27kDa protein, myosin-7 and MYH1 protein were up-regulated expression in yak LL muscle compared to cattle.

The Correlation of Differentially Expressed Proteins and Meat Quality

The 7 target proteins (MLC, HSP 27, TIM, KRT 10, LGALS1, GAPDH and HBA) were screened in our study, they could play an important role in WHC of Yak and Cattle meat *(Table 7)*. The results showed that TIM had significant negative correlation with WBSF, HSP 27 had significant positive correlation with WBSF, and GAPDH had significant positive correlation with pH0h ($P<0.05$), those three proteins could be used the marker as tenderness. HBA was down-regulated during postmortem aging, and it had significant negative correlation with a* and L* ($P<0.05$). LGALS1 and KRT 10 were often considered as hypoxia-inducible factor. Baes on our analysis, we could presumptively use HBA, LGALS1 and KRT 10 as a meat color marker. MLC and HSP 27 had significant

Table 6. *Fold change of yak Vs cattle at 24 h in differentially expressed proteins*

Spot No.	Yak	Cattle	Protein Name
1958	-3.11075	3.11075	Keratin 10
899	2.92031	-2.92031	Myosin-2
1597	-2.27384	2.27384	Troponin T
1975	2.21049	-2.21049	Heat shock 27kDa protein
1883	-1.96894	1.96894	Thioredoxin-dependent peroxide reductase
1015	-1.88184	1.88184	ATP synthase subunit alpha
2028	-1.85099	1.85099	KRT4 protein
1486	1.83274	-1.83274	Myosin-7
1891	-1.67549	1.67549	Myosin, light chain 6B
1242	-1.60325	1.60325	Elongation factor Tu
1432	1.56064	-1.56064	MYH1 protein
1369	-1.53975	1.53975	Actin, alpha
1601	-1.38845	1.38845	Myozenin-1

Table 7. *the correlation of differentially expressed proteins and meat quality*

Protein Name	pH_{0h}	pH_{24h}	a*	b*	L*	WBSF	WHC
TIM	0.59	0.56	0.22	0.21	-0.23	-0.68*	0.13
HSP 27	0.41	0.33	0.03	0.15	-0.04	0.84*	0.80*
KRT 10	0.04	0.39	0.85*	0.11	0.08	0.37	-0.76*
MLC	0.34	0.32	0.41	0.18	0.29	0.36	0.91*
LGALS1	0.06	0.36	0.82*	0.12	0.11	0.29	0.27
GAPDH	-0.79*	0.29	0.35	0.19	0.26	0.42	0.52
HBA	0.04	0.39	0.87*	0.07	0.06	0.49	0.36

Bioinformatics Analysis

Three structured terms (cellular component, molecular function and biological process) are widely used in proteome, metabolome, transcriptome and genome research and according to similarity properties, we grouped our proteins under these categories *(Fig. 2)*.

Functional enrichment analysis was conducted on all differentially expressed proteins by blast2GO 2.8 software [24]. As for GO term enrichment of cellular components, proteins related to Cell, Organelle, Extracellular region, Macromolecular complex and Membrane were the top 5 enriched. Similarly, as for GO term enrichment of molecular function, proteins related to Binding, Catalytic, Structural molecule, Transporter and Electron carrier were the top five enriched. Proteins involved in biological processes, such as Single-organism process, Cellular process, Metabolic process, Multicellular organismal process and Biological regulation, were found to be the top 5 enriched.

We used the KEGG database to understand the key role of each protein identified in our database [25] *(Fig. 3)*. It showed that proteins differentially expressed were significantly enriched in multiple biological processes, including carbon metabolism, gluconeogenesis, biosynthesis of aminoacids, pyruvate metabolism, TCA cycle, oxidative phosphorylation and MAPK signaling pathways *(Fig. 4)*.

DISCUSSION

Characteristics of Meat Quality

Myoglobin is the principal pigment responsible for the redcolor of meat, and may increase reflectance providing a

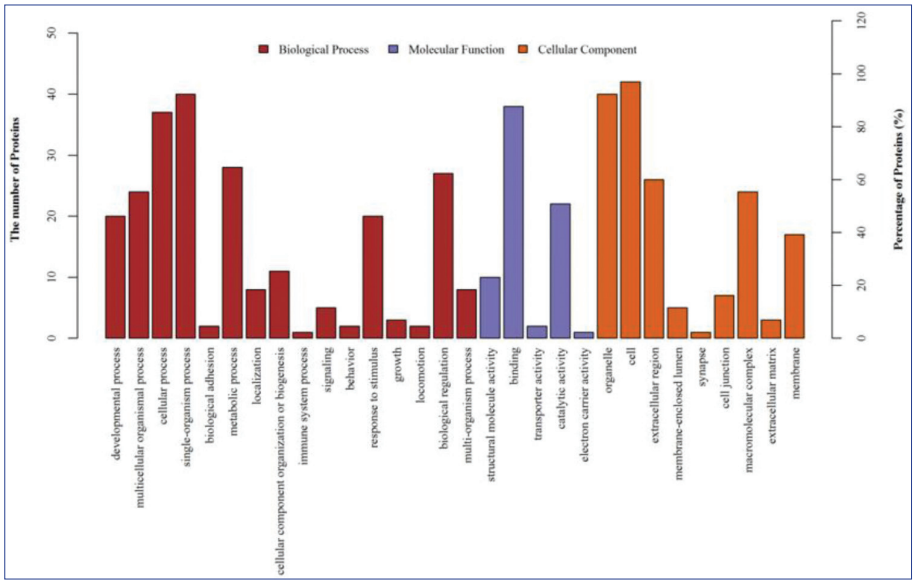


Fig 2. Classification of differential expression proteins identified by GO functional classification

negative correlation with WHC, and KRT 10 had significant positive correlation with WHC (P<0.05), all these findings highlight the importance of MLC, HSP 27 and KRT 10 in WHC.

lighter appearance and increased yellow coloration related to the increased free water content of tissue surface [26]. Yak was significantly higher than cattle in meat redness (a*)

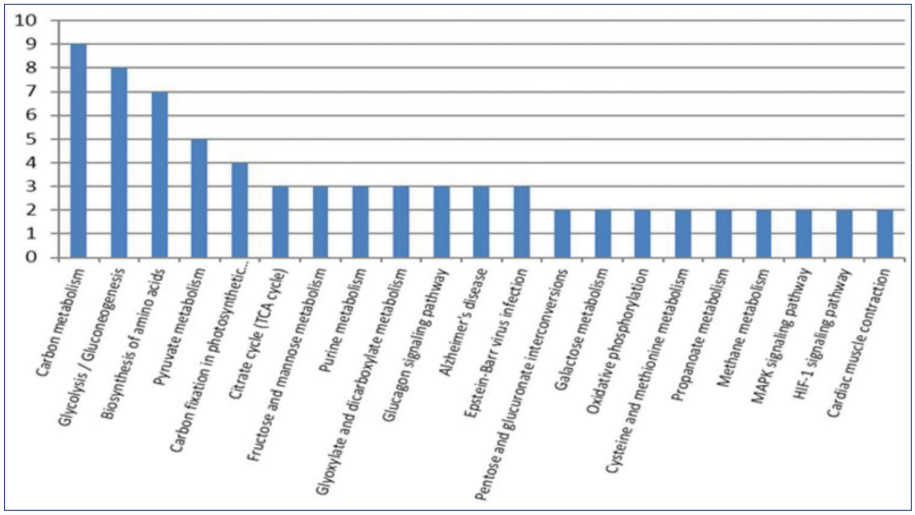
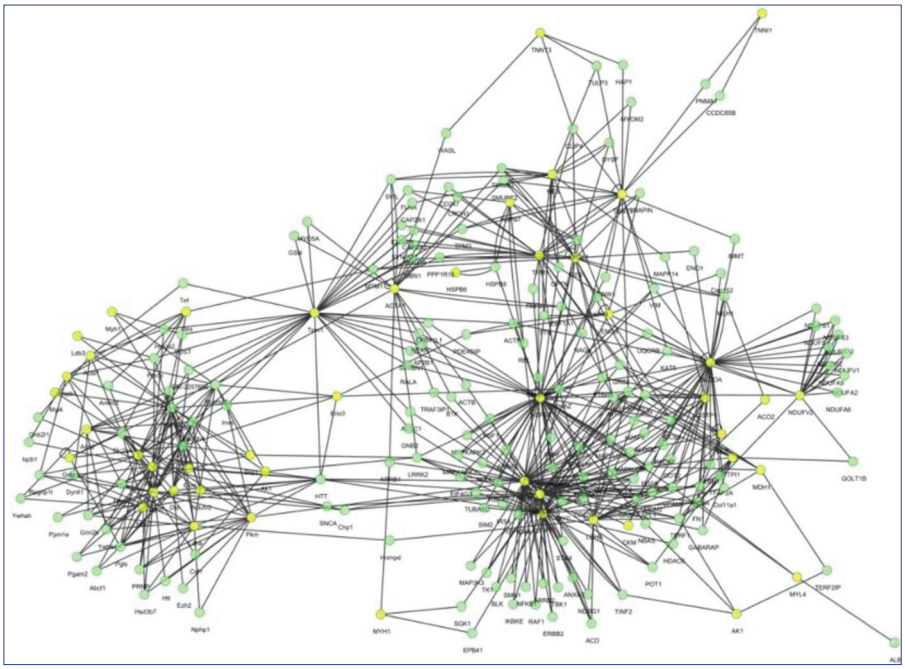


Fig 3. Classification of differential expression proteins identified by KEGG pathway enrichment analysis

Fig 4. Protein-protein interaction networks of the differential abundance proteins of Yak and Cattle groups in LL muscle based on Cytoscape 3.0 software. The nodes were proteins from Bos Taurus database and the lines were the predicted functional annotations (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)



value, and was significantly lower than cattle in lightness (L; $P<0.05$). There were no significant difference in yellowness (b*) between yak and cattle ($P>0.05$). The result showed that the pH_{0h} was also strongly consistent in yak meat with surface color at 0 h after slaughter, which confirms that early postmortem pH might represent a significant quality predictor for meat color in both groups, as previously found [27].

Yak WBSF values and WHC value was significantly higher than cattle ($P<0.05$). Meanwhile, yak intramuscular fat content was significantly lower than cattle ($P<0.05$). Yaks have numerous physiological traits that equip them for life at high altitudes, including more myofibrillar and cytoskeletal proteins. It is well known that after examining some myofibrillar proteins degraded and declined in intensity, high-altitude hypoxia results in less tender in yak meat than cattle [28].

Categories of Proteins Identified in Yak and Cattle Tissue

It was well known that the proteolytic degradation of structural proteins play a major role in increasing muscle moisture [29]. Structural proteins mainly included collagen, elastic protein and proteoglycan, which allow the binding of muscle tissue to other cellular material [30]. Once myofibrillar and cytoskeletal proteins are degraded during postmortem aging, muscle structures will become looser and muscle moisture content will descend [31]. Some myofibrillar and cytoskeletal proteins varied significantly between yak and cattle, namely Myosin and Keratin family proteins, Alpha-crystallin B chain, Troponin T and Actin [18].

Structural proteins are an important material component for cells and organisms. The myosin family of proteins are major structural protein associatedwith tubulin (such as actin); their soluble monomer can be made of long, rigid

fiber, and form the cytoskeleton by polymerization [32]. The solubility of some myosin proteins decreased and some myofibrillar proteins were fragmented and released during muscle aging [33]. Many scholars [34] showed that denaturation of the myosin could contribute to myofibrillar lateral shrinkage and reduced muscle WHC. Myosin light chain (MLC) proteins play an important role in forming muscle protease catalysis supramolecular complexes, and MLC could catalyze phosphorylation by MLCK which is a type of calmodulin dependent enzyme. Actomyosin could activate myosin ATPase to cause smooth muscle contraction activity [35]. MLC provided an important contribution to the structure and tensile strength of meat, and expression was up-regulated after 24 h of aging in yak and cattle. The result was that muscles need to improve the anaerobic energy metabolism for the supply of ATP during postmortem aging. This conclusion was similar to Jia [36] who found myosin family proteins could accelerate the systolic shrink of muscle fibers in overgrowth phenomenon of cattle. Therefore, MLC may act as a potential biomarker of WHC related to meat aging.

Keratin family, Alpha-crystallin B chain and Troponin T proteins could inhibit the actomyosin ATPase activity, and protect the overall integrity of muscle cells, and once those proteins are degraded, the integrity of meat myofibril could be disrupted [28]. In recent years, more and more study showed keratin 4 has more complex functions in cell growth, translation process, cell proliferation, organelle transport and stress response and protection. This study results showed that KRT4 has a significant positive correlation with a* value, we speculated that it played an important role in protecting cells against external environmental stress and injury.

When the animals were killed, it would stop their circulation of blood and cut off the oxygen supply [33]. The muscles can no longer remove metabolic products, leading to lactic acid accumulation by glycolysis of muscle glycogen. Muscle pH then lowers compared to within the than living body, meanwhile, myosin and actin were linked, the WHC of muscle was reduced and calcium was released [37]. In our study, eight kinds of proteins in metabolic enzymes *(Table 2)* were found to be present in different abundance comparing yak and cattle tissue. Thioredoxin-dependent peroxide reductase, Triosephosphate isomerase, ATP synthase subunit alpha, Glyceraldehyde-3-phosphate dehydrogenase, Flavin reductase (NADPH) were the key proteins for glycolysis and tricarboxylic acid cycle, in which it catalyzes the interconversion of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate, and it is essential for efficient energy production.

Through the research in recent years, it was shown that differences of protein expression in tender meat mainly included structural proteins, metabolic enzymes and stress related proteins [24,38]. The mechanism was that high glycolysis rate contributes to lactate accumulation, and the decrease of pH reduced the ions repulsion between the myofibrillar proteins and it could decrease the repulsion between filaments of myofibrillar, which contributed to lateral shrinkage of the muscle fibres, then sarcomere extension, and lead to higher WHC [39].

The study found that high degree of tender meat had a relatively high of Thioredoxin-dependent peroxide reductase, it could be used as a prediction of protein hydrolysis and tender meat of marker protein in beef. At the same time, the expression quantity of Triosephosphate isomerase and ATP synthase subunit alpha decreased during refrigeration. The active site of TIM was glutamate residues, when it bind to substrate, the catalytic reaction is quickly activated, and this reaction could accelerate meat tenderness.

Previous research found that the expression of stress related proteins could increase in hyperthermia, hypoxia, nutritional deficiency, oxidative stress, ultraviolet radiation and reperfusion following ischemic injury [40]. The expression and phosphorylation of HSP 27 could be up-regulation and prevent structural damage and degradation of proteins under the pressure. HSP 27 and Daxx could inhibit the apoptosis which was mediated by Fas. Apoptosis was closely related to shrinkage and water loss of cells. HSP 27 would inhibit the release of C cytochrome to prevent the expression of pro-apoptotic protein, C cytochrome regulates TNF-α and Fas ligand to induce apoptosis, so HSP27 would have effect on WHC by regulating cell apoptosis and have potential as biomarkers for moderate to good WHC [41].

Early thermo resistance was mainly influencing cytoskeleton, it could break actin filaments and resolve microtubules [26]. HSP 27 participated in thermo resistance process by regulating the polymerization of actin, and it was necessary to structural stability of actin microtubules in HSP 27 phosphorylation. The abundance of both the stress-induced thioredoxin-dependent peroxide reductase and glyceraldehyde-3-phosphate dehydrogenase was higher in 24 h than 0 h of yak and cattle meat. Thioredoxin-dependent peroxide reductase and Glyceraldehyde-3-phosphate dehydrogenase played a role in mechanisms of cellular detoxification and cellular resistance to oxidation, and under the condition of oxidation, the expression quantity of HSP 27 phosphorylation was up-regulation, and the affinity of actin poly was weaken, then HSP 27 was dissociation from actin poly, and the actin monomers enhanced the binding force with microtubules, and improved the resilience on oxidation of actin microtubules. All these findings highlight the importance of stress and related proteins in the proteomic response associated with WHC [42].

Our study, compared to cattle at 0 h, hemoglobin alpha was identified showing a lower abundance in 24 h compared to 0 h of both cattle and yak. Studies had shown that

Hemoglobin alpha had strong ability of the combination to nucleotide, so we think that Hemoglobin alpha could play a role in nucleotide carrier for muscles, meanwhile, the expression quantity of Hemoglobin alpha would be greatly up-regulated under oxidation, and it also could play a role in cell growth and development by the pathway of cell mitochondrial synthesis and transport [43]. Results of the aforementioned studies indicated the need for further research on the role of this protein in yak and cattle meat.

The meat color was determined by concentration and chemical state of Hemoglobin alpha [44]. Yak increase the Hemoglobin alpha content of meat to meet the demand for oxygen, so the yak meat had a darker red color. In our study, we showed that the content of Hemoglobin alpha had positive correlation with a*.

Binding proteins are a type of glycoprotein macromolecule, commonly found in Animal tissues, which play an important role in many physiological and pathological process, such as cell adhesion, apoptosis, inflammatory response and neoplasm metastasis [45].

In Doutaud's study, the expression of Galectin-1, which is a type of hypoxia regulatory proteins, was up-regulated in a hypoxic environment, possibly to promote the growth of vascular tissue. On the contrary, the expression of Galectin-1 was invariant in normal tissue. We could use Galectin-1 as the marker of hypoxia. In our study, there were Galectin-1 in yak and cattle meat and we speculate that the phenomenon could be associate with the low oxygen environment in which both animals live.

Proteins serve as fundamental parts of protein complexes in living cells, and adjust and mediate by other proteins to carry out shared functions. It was important for revealing the function of the protein to explore the protein-protein interaction networks [36]. Some proteins acted as core proteins as shown by gene name in biological interaction networks (BIN), such as MLC, HSP 27, TIM, KRT 10, LGALS1, GAPDH and HBA *(Fig. 4)*. In BIN, gene names of differentially expressed proteins could be summarized into two major categories. One showed the enzymes of glycolytic and energy metabolism, and the other was cell structure.

This study provides a better understanding of proteome changes in yak and cattle muscle.

HSP 27, MLC and KRT 10 indicate differential expression patterns between yak and cattle groups after 0 h and 24 h of postmortem aging. The bioinformatics results showed that differential expressed proteins involved in glycolytic and energy metabolism enzymes and structural proteins. Further studies about post-translational modifications and the changes in metaboliteof the related proteins remain to be explored. The functions of the identified proteins contribute to a more detailed molecular view of the processes behind WHC and are a valuable resource for future investigations.

Conflicts of Interest

The authors declare no conflict of interest.

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