Analysis of the molecular mechanism of inosine monophosphate deposition in Jingyuan chicken muscles using a proteomic approach

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ABSTRACT Inosine monophosphate (**IMP**) is an indicator of meat taste, and the molecular mechanism underlying IMP deposition in muscle tissues is important to developing superior poultry breeds. The aim of this study was to identify the key proteins regulating IMP deposition in different muscle groups of 180-day-old Jingyuan chickens (Hen) using a proteomics-based approach. We identified 1,300 proteins in the muscle tissues of Jingyuan chickens, of which 322 were differentially expressed between the breast and leg muscles (129 proteins were highly expressed in breast muscles and 193 proteins were highly expressed in leg muscles). PGM1, PKM2, AK1, AMPD1, and PurH/ATIC were among the differentially expressed proteins (**DEPs**) involved in the purine metabolism pathway, of which purH was highly expressed in leg muscles, while the others were highly expressed in breast muscles. The proteomics screening results were verified by PRM, qPCR, and western blotting, showing consistency with the proteomics results. Our findings are not only significant in terms of protecting the Jingyuan chicken germplasm resources, but also provide the molecular basis for generating high-quality broiler chicken breeds.

Key words: Jingyuan chicken, breast muscle, leg muscle, proteomics, IMP

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INTRODUCTION

Chicken is the most commonly consumed meat worldwide and is rich in protein. Although genetic engineering has significantly improved the growth rate and muscle yield of chickens relative to feed consumption (Buzón-Durán et al., 2017; Zhichao et al., 2019), the quality of the meat has declined (Lodens et al., 2020). Therefore, a major concern for the broiler industry is to improve meat quality while maintaining yield (Stadig et al., 2016).

Meat quality is an economically important trait, and is evaluated in terms of the umami taste, flavor, texture, nutrition, and safety, among other factors. Umami is determined by inosine monophosphate (**IMP**), an intermediate product of nucleotide metabolism with a 40-fold higher umami taste compared to sodium glutamate (**MSG**). IMP content is, therefore, an important indicator of meat quality and freshness (Blonde and Spector, 2017; Gabriel et al., 2018). The IMP content differs across the different muscles, which affects broiler production and further processing. However, the molecular mechanism underlying the site-specific deposition of IMP in chickens is still unclear. It is essential to identify the key proteins that regulate differences in IMP deposition in chicken muscles in order to improve meat quality and breed novel poultry varieties.

Proteomics, or the analysis of the entire protein complement of a cell, tissue, or organism under specific conditions (Hyung and Ruotolo, 2012; Oeckl et al., 2015; Suraj et al., 2019), is increasingly being used in poultry research. For example, Parada et al., analyzed the molecular mechanisms of neurogenesis in chicken embryos through cerebrospinal fluid proteomics. Likewise, Teltathum and Mekchay (2009), identified key functional proteins in Thai chickens at different growth stages, and other groups identified the characteristic proteins of the egg shell, egg white, yolk, and yolk membrane (Mann et al., 2006; D'Ambrosio et al., 2008; Mann, 2008; Farinazzo et al., 2009). Likewise, O'Reilly et al. used the proteomics approach to study the intestinal microorganisms in broiler chickens and found that actin and its related proteins gradually increased over time, while antiapoptotic and heat shock proteins showed a time-dependent decline. Schilling et al. found that compared to normal chicken meat, there were 15 differentially expressed proteins in pale, soft, and

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exudative (**PSE**) meat and eight in woody meat, indicating that specific protein expression is related to meat quality. Fu et al. (2013) identified ACAA2, ANXA2, GYGI, LOC418811, MDHI, and PFK as the key proteins regulating muscle development and lipid metabolism in the Beijing oil chicken (Fu , 2013). Thus, the protein complement of chicken muscle depends not only on the growth stage and muscle type, but also on the environmental conditions and species characteristics.

The local broiler breeds in China are characterized by high meat quality, disease resistance, and adaptability. Jingyuan chicken, one of the 5 district-level livestock and poultry germplasm resources in the Ningxia Autonomous Region, is an excellent broiler breed listed in the China Animal and Poultry Genetic Resources Catalog. We identified the key proteins regulating IMP deposition in Jingyuan chicken muscles using an unlabeled and quantitative proteomics approach, and functionally annotated them through bioinformatics analyses. Parallel reaction monitoring (**PRM**), qPCR, and western blotting (WB) were then used to verify differences in the abundance of these proteins and mRNAs.

MATERIALS AND METHODS

Reagents

Sequencing-grade modified trypsin was purchased from Promega (Fitchburg, WI). Iodoacetamide (**IAA**), dithiothreitol (**DTT**), trifluoroacetic acid (**TFA**), EDTA, urea, and tetraethylammonium borohydride (**TEAB**) were obtained from Sigma (St. Louis, MO). Formic acid (**FA**) was purchased from Buches (Germany). Protease inhibitor cocktail III, TMT kit, can, and pure water was purchased from Thermo Fisher Scientific (Waltham, MA). The 2-D Quant Kit was obtained from GE Healthcare (Buckinghamshire, UK). TIMS-TOF Pro (Bruker, Germany) was used for LC-MS/MS. The RNA inversion kit (dp431) was purchased from Tiangen company (China). All qRT-PCR kits were purchased from Ecoray Biological Company (Seoul, South Korea). The protein extraction kit, BCA protein quantitation kit, skimmed milk powder, polyvinylidene difluoride (**PVDF**) membranes, SDS-PAGE gel preparation kit, PMSF, 10x protein loading buffer, Coomassie Brilliant Blue fast staining solution, developing, and fixing solution, ECL solution, and T protein marker were all purchased from Shanghai Wansheng Haotian Biotechnology Co. Ltd. (China). Goat anti-rabbit AMPD1 antibody (nbp2-24508; Novus Biologicals, Littleton, CO) and rabbit anti-mouse GAPDH antibody (sc-293335; Santa Cruz Biotechnology, Dallas, TX) were used for western blotting. The primers were synthesized by Shanghai Shenggong (China).

Animals and Samples

Jingyuan chickens were provided by the Chaona Chicken Breeding Center in Pengyang County, Ningxia, of which 150 white feathered hens that had been reared for 180 d and weighed 2.5 ± 0.23 kg were selected for slaughter. The leg muscles and breast muscles were dissected and crushed, and snap frozen at −80°C. All experiments were conducted according to the Animal Care and Use Guidelines of the Animal Care Committee of Ningxia University in China.

Determination of IMP

IMP was extracted from muscle tissues of Jingyuan chickens using the "determination of creatinine content in yellow feather broiler product quality classification standard" (GB/ t19676-2005). The mobile phase of liquid chromatography was ammonium formate solution, and the UV detection wavelength was 254 nm.

Protein Extraction

The frozen muscle samples were pulverized in liquid nitrogen and homogenized on ice with 4 volumes of lysis buffer (8 M urea, 1% Triton-100, 10 mM dithiothreitol, and 1% protease inhibitor cocktail) in a high-intensity ultrasonic processor (Scientz, Ningbo, China). The samples were sonicated 3 times, and centrifuged at 20,000 *g* for 10 min at 4°C. The proteins were precipitated with 20% TCA at −20°C for 2 h, and centrifuged at 12,000 *g* for 10 min at 4°C. The supernatant was discarded, and the precipitate was washed three times with cold acetone. The protein was dissolved in 8 M urea and quantified using the BCA kit according to the manufacturer's instructions.

Trypsin Digestion

The protein samples were diluted in 5-mM dithiothreitol and reduced at 56°C for 30 min. After incubating with 11 mM iodoacetamide at room temperature in the dark for 15 min, the urea concentration in the samples was diluted to below 2 M. Pancreatin was added at the mass ratio of 1:50 to the protein, and digested overnight at 37°C. The mass ratio was decreased by 1:100 and digested for a further 4 h.

Liquid Chromatography-Mass Spectrometry

The peptides were suspended in liquid chromatography mobile phase A (0.1% v/v formic acid solution) and separated using the nanoElute (Bruker) ultra-high-performance liquid system with the following liquid phase gradient: 0 to 70 min, 6% ~ 22% B (1% formic acid in acetonitrile); 70 to 84 min, 22% ~ 32% B; 84 to 87 min, 32% ~ 80% B; 87 to 90 min, 80% B. The flow rate was maintained at 300 nL/min. The eluted peptides were injected into the capillary ion source for ionization and were analyzed by timsTOF Pro mass spectrometry. The ion source voltage was set to 1.4 KV, and the peptide precursor ion and its secondary fragments are detected and analyzed using TOF. The secondary MS scan range was set to 100 to 1,700 m/z. Data was acquired in the parallel cumulative serial fragmentation (**PASEF**) mode. After primary mass spectrometry, the 10 times PASEF mode was used to acquire the secondary spectra with a precursor ion charge in the range of 0 to 5. The dynamic exclusion time of the tandem mass spectrometry scan was set to 24 s to avoid repeated scans of the parent ion.

Database Search

The secondary mass spectrometry data were retrieved using Maxquant (v1.6.6.0) from the *Gallus*_ *gallus*_UniProt database. An anti-library was added to calculate the false positive rate (**FDR**) caused by random matching, and a common pollution library was added to eliminate the contamination of the protein impact. The digestion method was set to Trypsin/P, number of missed cut sites to 2, mass error tolerance of the primary precursor ion to 70 ppm for both the first and main search, and the mass error tolerance of the secondary fragment ion to 0.04 Da. The cysteine alkylation was set as fixed modification, and the variable modification as methionine oxidation, acetylation, and deamidation of protein N-terminus. The false discovery rate (**FDR**) for protein identification and the peptide-spectrum match (**PSM**) was set to 1%.

Bioinformatics and Statistical Analyses

The differentially expressed proteins were annotated by Gene Ontology (**GO**), Kyoto Encyclopedia of Genes and Genomes (**KEGG**) pathway, and protein domain analyses. The functional categories that were significantly enriched (P-value < 0.05) in at least one protein group were selected, and the data matrix was logarithmically transformed. The -log10 data were applied to each function classification by Z transformation, followed by hierarchical clustering (Euclidean distance, average connection clustering) for unilateral cluster analysis. The heatmap was drawn using the function heatmap.2 in the R package "gplots".

PRM

PRM was used to quantify the candidate biomarker proteins of breast and leg muscles by LC-PRM/MS. The peptide information was imported into Xcalibur software for PRM calibration. Briefly, 10 μg of each peptide sample was mixed with 200 fmol standard peptide (Pierce retention time calibrator [**PRTC**]: TASEFDSAIAQDK) in mobile phase A (0.1% v/v formic acid and 2% acetonitrile) and separated using the EASY-nLC 1000 ultra-high performance liquid system. The following liquid gradient was used: 0 ~ 40 min, 6% -25% mobile phase B (0.1% formic acid and 90% acetonitrile); 40 ~ 52 min, 25 to 35% B; 52 ~ 56 min, 35 to 80% B; 56 ~ 60 min, 80% B. The flow rate was maintained at 400 nL/min. The eluted peptides were injected into the NSI ion source for ionization and analyzed by Q exactive plus mass spectrometry. The voltage of the ion source was set to 2.0 kv, and the peptide parent ions and their secondary fragments were detected and analyzed by high-resolution Orbitrap (Thermo Fisher). The scanning range of primary mass spectrometry was set to 390 to 1,100 M/Z, the scanning resolution to 70,000, and the scanning resolution of secondary mass spectrometry Orbitrap to 17,500. Data were acquired with the data independent scanning (**DIA**) program, and the fragmentation energy of the HCD collision pool was set to 27. The AGC was set to 3e6, the maximum to 50 ms, AGC to 1e5, the maximum to 150 ms, and the isolation window to 1.6 m/z. The original PRM file was analyzed using Skyline 3.5.0 software.

Real-Time qPCR

Frozen leg and breast muscle tissues were homogenized in liquid nitrogen, and total RNA was extracted using the RNA extraction kit (Tiangen DP431), according to the manufacturer's instructions, and was reverse-transcribed to cDNA. The primers for AMPD1, PGM1, PKM2, GAPDH, and β-actin were designed using Primer Premier 5.0 software based on the published sequences of chicken AMPD1 (accession number XM_003642728), PGM1 (accession number NM_001038693.2), PKM2 (accession number: XM_015278795.2), GAPDH (accession number NM_204305.1), and β-actin (accession number: NM_205518.1). The sequences are summarized in Table 1. The SYBR Green Pro Taq HS kit was used for qRT-PCR reactions, and the relative gene expression levels were calculated using the $2^{-\triangle\triangle Ct}$ method.

Western Blotting

Total protein was extracted from the frozen muscle tissues using a specific kit according to the manufacturer's instructions and quantified. Equal amounts of proteins per sample were separated by SDS-PAGE, and the protein bands were transferred to PVDF membranes for WB as per standard protocols.

Data Analysis

SPSS version 25.0 (IBM Corp., Armonk, NY) was used for data analysis. The data were expressed as means ± standard deviation, and the control and experimental groups were compared by random one-

Table 1. Primers information of real-timePCR.

Gene	Accession	Primer sequence information
AMPD1	XM_003642728	F:TACCCAGGATTTATGATGT R:CTTGAGGATTGACAGTTG
PGM1	NM_001038693.2	F:ATCACTGGCAGAAGTATGG R:CAAAGGAGCGGTCAA
PKM2	XM_015278795.2	F:GTGTTCGCTTCCTTCATC R:ATTCTCAATCTTGCTGATAATCT
GAPDH	NM_204305.1	F:CTGTCAAGGCTGAGAACG R:GATAACACGCTTAGCACCA
ACTIN	NM_205518.1	F:TGCGTGACATCAAGGAGAAG R:GGACTCCATACCCAAGAAAGAT

Table 2. Determination results of IMP content in breast and leg muscle tissues of Jingyuan chicken.

Tissue sample	XJ1	XJ2	XJ3	XJ4	XJ5	XJ6	TJ1	TJ2	TJ3	TJ4	TJ5	TJ6
IMP content	3.73^{a}	3.63^{a}	2.86^{a}	1.29^{a}	1.27^{a}	2.34^{a}	1.24^{b}	1.14^{b}	0.95^{b}	0.17^{b}	0.32^{b}	0.63^{b}

Note: "XJ" stands for Jingyuan chicken breast muscle tissues, and "TJ" stands for Jingyuan chicken leg muscle tissues.
Abbreviation: IMP, inosine monophosphate.
^{ab}Different lowercase letters in the same set of data indicate extremely significant differences ($P < 0.01$).

way ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS

IMP Deposition in the Muscle Tissues of Jingyuan Chickens

The IMP content of Jingyuan chicken breast muscle (**XJ**) and leg muscle (**TJ**) was measured by liquid chromatography, which indicated significantly higher amounts in the breast muscle compared to the leg muscle (Table 2).

Peptide Length Quality Control for Breast and Leg MuscleTissue Proteins

As shown in the SDS-PAGE gel image in SI Appendix, Figure S1, the proteins extracted from the leg and breast muscles were intact and showed consistent bands. The proteins were further analyzed by LC-MS, and the amino acids and peptides were mapped to the "proteomes gallus (chicken)" database. Most peptides were 7 to 20 amino acids in length (SI Appendix, Figure S2), which is consistent with trypsin enzymatic hydrolysis and HCD fragmentation mode. Peptides shorter than 5 amino acids were considered too fragmented for sequence identification, whereas the mass and charge of peptides longer than 20 amino acids were too high for the fragmentation mode of HCD. Therefore, the peptide length identified by MS met the quality control requirements. Furthermore, the molecular weight of 98% of the peptides was less than 100 kDa (SI Appendix, Figure S3A). Since molecular weight correlates inversely with coverage density, this showed that the MS could be read and analyzed. Finally, 98% of the peptides had an error within ±10 ppm (SI Appendix, Figure S3B), which was in line with the quality control requirements.

Principal Component Analysis and Correlations of Leg and Breast Muscle Proteins

Principal component analysis (**PCA**) showed considerable differences between the breast muscle and leg muscle proteins (SI Appendix, Figure S4A), which is suggestive of distinct functions and metabolic pathways of the two muscle groups. To assess the reliability of the experiment, we calculated the correlation between the protein expression levels of the different samples, as shown in SI Appendix, Figure S4B. The Pearson correlation coefficient (R2) of the breast muscle and leg muscle tissue was greater than 0.81, indicating a high degree of similarity between the samples, and the general reproducibility of the experiment.

Screening and Identification of Differentially Expressed Proteins Between Breast and leg Muscles

LC/MS analysis of the peptides from the muscle tissues revealed 265,391proteins. This corresponded to the accurate identification of 14,689 peptides, including 12,830 specific peptides. Further matching and alignment with available peptide sequences identified 1,926 proteins, of which only 1,300 were verified quantitatively (SI Appendix, Figure S5). The differentially expressed proteins (**DEPs**) between the breast and leg muscles were screened using fold change > 1.5 or < 1.5 and *P*-value < 0.05 as the thresholds. A total of 322 proteins were differentially expressed between the breast and leg muscles, of which 129 were upregulated and 193 were downregulated, as shown in Figure 1. The DEPs

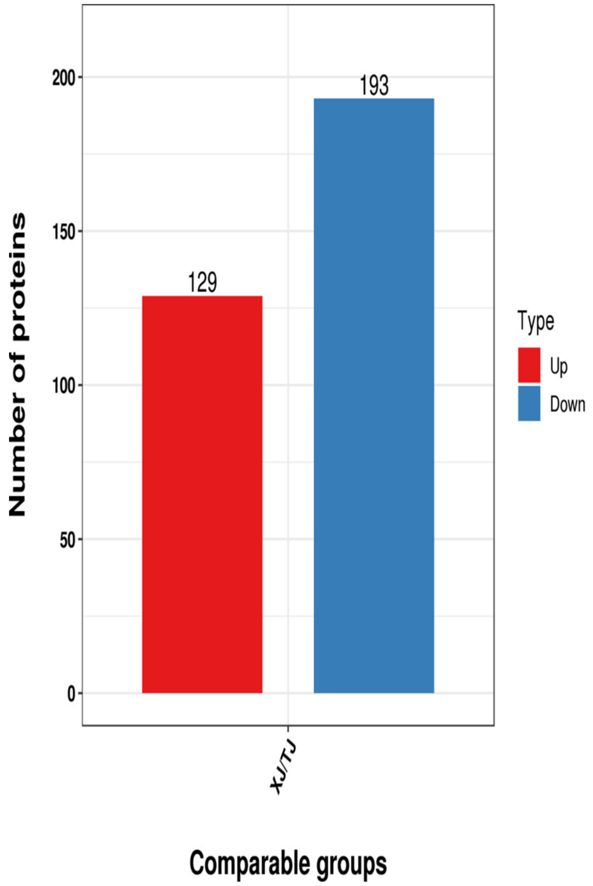

Figure 1. Results of statistical analysis of differential proteins in Jingyuan chicken breast and leg muscles.

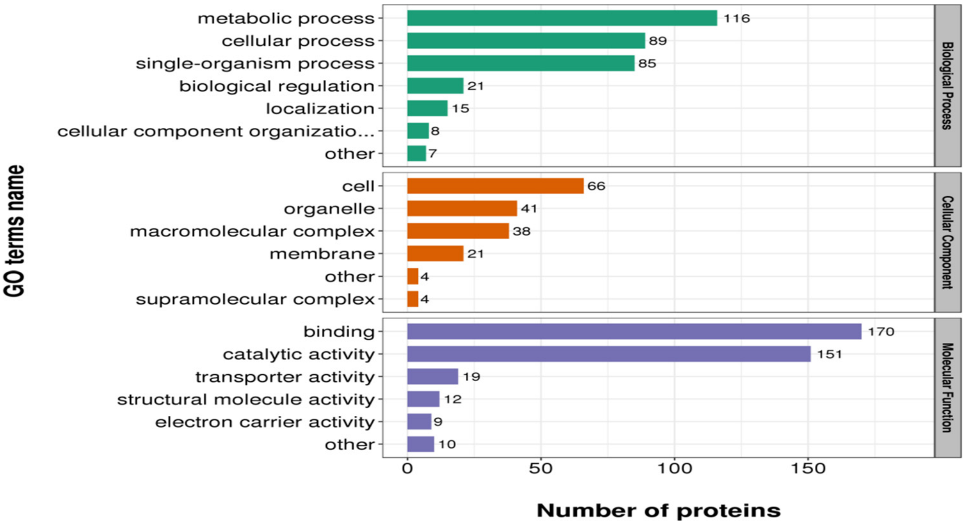

Figure 2. Differentially expressed proteins in breast and leg muscle tissues of Jingyuan chicken GO classification annotation. Abbreviation: GO, gene ontology.

accounted for 24.8% of the total number of quantitatively identified proteins, which further underscored the biological differences between the two muscle groups. The volcano plot of the DEPs is shown in SI Appendix, Figure S6.

Functional Annotation of the DEPs Between Breast Muscle and Leg Muscle

GO, KEGG pathway, clusters for original groups (**COG**), and subcellular structural localization were used to functionally annotate the DEPs. GO analysis showed that most DEPs were enriched in the "molecular function" group followed by "biological processes" (Figure 2), which is consistent with the results so far. Furthermore, COG/KOG annotation of the DEPs showed that most were clustered in the "energy generation and conversion" category, followed by "cytoskeleton" (Figure 3). The subcellular distribution of the DEPs was analyzed using the BRENDA, UniProt, and UniProtKB databases, which indicated that most proteins were localized in the cytoplasm and mitochondria. In addition, 17 DEPs were present in both the cytoplasm and nucleus (Figure 4). Finally, KEGG analysis showed that the DEPs are involved in 98 signaling pathways. As shown in the Figure 5, most DEPs were enriched in the oxidative phosphorylation pathway (gga00190).

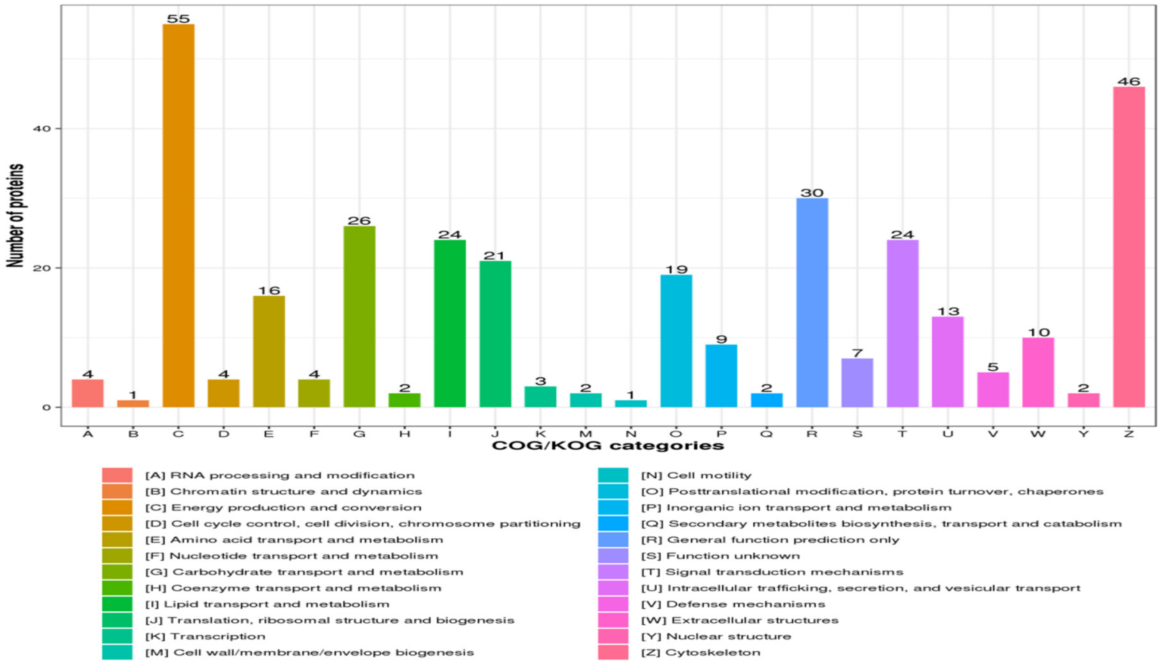

Figure 3. COG/KOG annotation analysis of differentially expressed proteins in breast and leg muscle tissues of Jingyuan chicken.

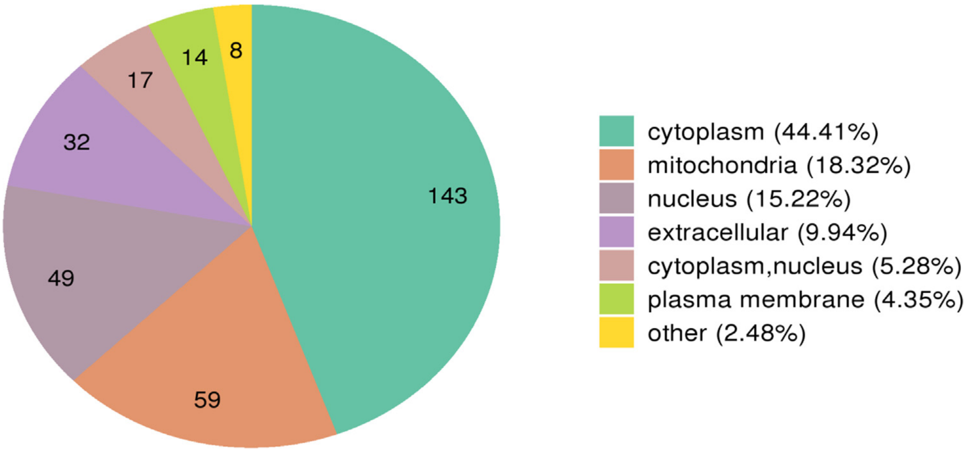

Figure 4. Analysis of subcellular structure location of differential protein in Jingyuan chicken breast and leg muscles.

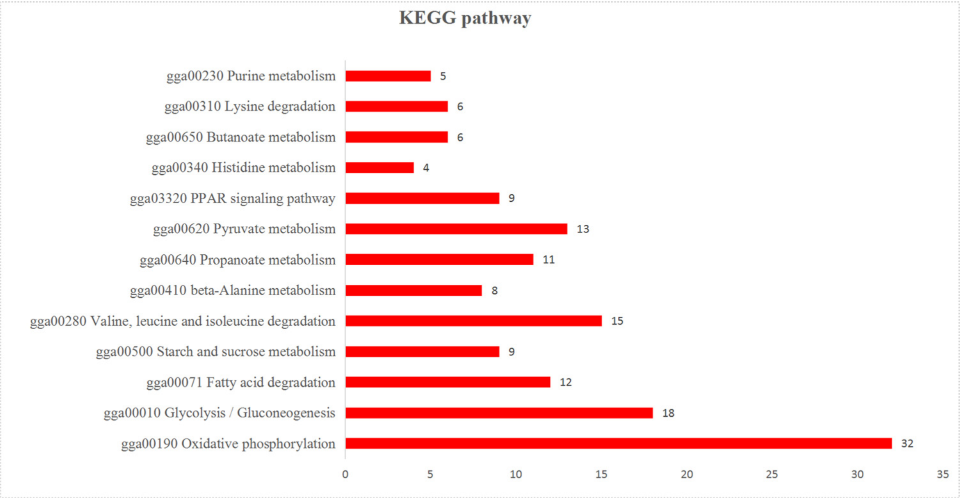

Figure 5. Differential protein KEGG pathway enrichment analysis results in breast and leg muscle tissues of Jingyuan chicken. Abbreviation: KEGG, Kyoto Encyclopedia of Genes and Genomes.

Screening and Identification of Key Proteins Regulating IMP Deposition

IMP is a by-product of the purine metabolism pathway (gga00230 Purine metabolism), which is activated in the muscle tissues. Five DEPs were enriched in the purine metabolism pathway, of which AMPD1, PKM2, PGM1, and AK1 were upregulated in breast muscle, whereas PurH/ATIC was highly expressed in the leg muscles. As shown in Figure 6, these proteins are directly or indirectly involved in the synthesis and catabolism of IMP, and therefore may have a key regulatory role in the deposition of IMP in the breast and leg muscles of Jingyuan chickens. Network analysis of these proteins using STRING and Cytoscape software indicated a functional relation among them, which, however, was not completely consistent with their KEGG pathways. As shown in Figure 7, PGM1 directly interacted with PKM2 protein in the IMP metabolism pathway, although the specific molecular mechanisms need to be studied further. The key IMP-related proteins were quantitatively verified by comparing the LC and MS results using PRM. As shown in SI Appendix, Figures S7−S11 and Table 3, the results of the two experiments were similar.

Verification of Key Proteins Regulating IMP Deposition

The key proteins related to IMP deposition in the muscle were further verified by qRT-PCR and western blotting. As shown in Figure 8, the expression levels of AMPD1, pkm2, and PGM1 mRNAs were significantly higher in the breast muscles compared to the leg muscles, which was consistent with proteomics results and PRM identification. Therefore, the differential expression of these proteins in the two muscle groups was consistent with the transcription of their coding genes. Finally, western blotting of three biological replicates of breast and leg muscles verified that the AMPD1 protein was expressed at significantly higher levels in the breast muscle compared to the leg muscle (Figures 9A and 9B).

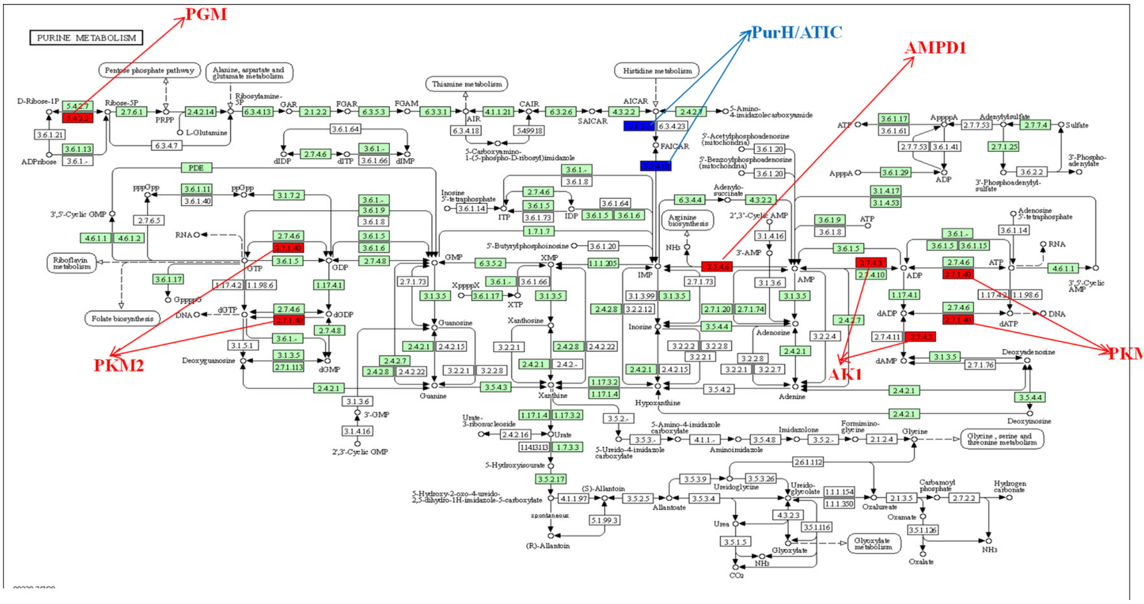

Figure 6. Visual screening and identification results of key proteins that regulate the specific deposition of IMP in Jingyuan chicken breast and leg muscle tissues. Abbreviation: IMP, inosine monophosphate.

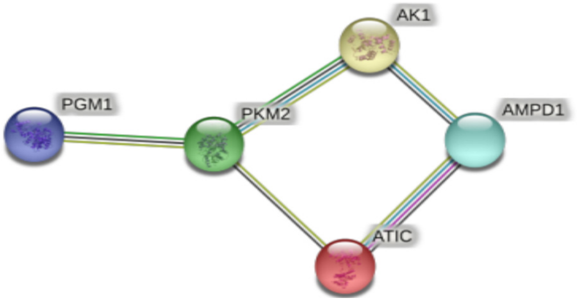

Figure 7. Interaction analysis of key proteins that regulate the specific deposition of IMP in the breast and leg muscles of Jingyuan chicken. Abbreviation: IMP, inosine monophosphate.

DISCUSSION

The yield and quality of chicken meat are closely related to the live weight and carcass weight, and 40 to 50% of the latter consists of the breast and leg muscles. Although both muscles originate from the same segments, they develop through different myogenic pathways (Mok and Sweetman, 2011), which translates to distinct biological, physical, and biochemical characteristics. For instance, the breast muscle primarily consists of white muscle and intermediate fibers, while the leg muscles are largely made of red muscle fibers with some white muscle and intermediate fibers (Liu, 2009; Wang et al., 2015). In addition, the muscles also differ significantly in terms of muscle fiber diameter and density (Liu et al., 2006; Liu, 2009). The leg muscles show higher pH (Tang et al., 2006), lower luminance (Jia et al., 2008), greater shear force (Shi and Wanping, 2001), less drip water loss, greater nutrient loss during steaming (Yang et al., 2012), higher crude fat content, lower protein, and amino acid content (Li et al., 2003), and higher intramuscular fat (**IMF**) compared to the breast muscles. In addition, the amount of dry matter, cholesterol, and certain minerals and vitamins also differ considerably between these muscles. Finally, leg muscles have only 30% of the amount of IMP − the important factor affecting meat flavor (Hayabuchi et al., 2020) − present in the breast muscle (Liu et al., 2014). However, the molecular mechanisms underlying the spatial deposition of IMP in chicken muscle have not been elucidated so far. We identified 1,300 proteins in the Jingyuan chicken through proteomic sequencing, of which 129 were upregulated and 193 were downregulated in the leg muscles relative to breast muscles. The downregulated proteins were enriched in 13 KEGG pathways, including the purine metabolism

Table 3. Analyze and compare proteomics identification results with PRM quantitative verification results.

	LC and MS results		PRM results	
Protein gene	TJ/XJ Ratio (FLQ)	TJ/XJ *P*-value (FLQ)	TJ/XJ Ratio (PRM)	TJ/XJ P-value (PRM)
AMPD1	0.312	0.00183601	0.46	0.000670923254690344
AK1	0.325	0.0020765	0.42	0.00481775848031593
PKM2	0.211	0.000102127	0.26	0.00222837509674208
PGM1	0.203	0.0001151	0.29	0.00045073179048575
PurH/ATIC	2.76	0.0129312	2.82	0.0132068548245433

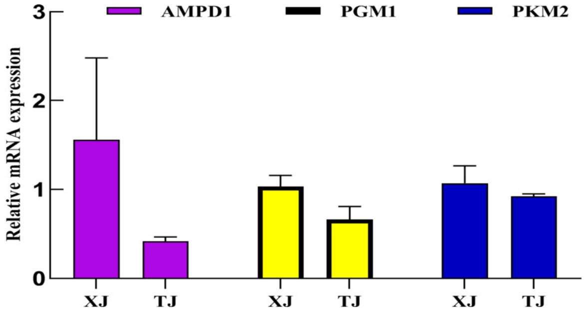

Figure 8. The results of qPCR verification of the mRNA expression of some proteins that regulate IMP-specific deposits in the breast and leg muscles of Jingyuan chicken.

pathway. In addition, 4 proteins involved in IMP deposition – AMPD1, AK1, PKM2, and PGM1 – were significantly downregulated in the leg muscles.

Adenosine monophosphate deaminase (**AMPD1**) catalyzes the hydrolysis and deamination of AMP to IMP and ammonium ions, and its activity is an indicator of cellular energy demand (Stratil et al., 2000). AMPD1 activation depends on the levels of intracellular metabolites, especially purine and inorganic phosphorus (Wheeler and Lowenstein, 1979). AMPD1 expression is highest in abdominal fat, followed by sebum, breast muscles, leg muscles, and liver, with relatively less in the heart, kidney, and stomach muscles, and is almost absent in stomach glands, spleen, and lungs. Consistent with this, the *AMPD1* gene and translated protein were found to be downregulated in the leg muscles of the Jingyuan chicken. In addition, the IMP content is positively correlated with AMPD1 expression in different chicken breeds (Chen Jilan, 2004), therefore, a potential marker for selecting chicken with high muscle IMP content and flavor.

Adenylate kinase 1 (**AK1**) is expressed in highly regenerative tissues such as skeletal muscles, the hematopoietic system, and brain (Tanabe et al., 1993), and regulates adenine nucleotide metabolism. AK1-deficient mice show normal muscle formation but delayed relaxation of skeletal muscle due to an excessive accumulation of ADP. AK1 phosphorylation also relays signals between the mitochondria and KATP channels (Carrasco et al., 2001). In a previous study, we found that AK1 levels affected the quality of the longissimus dorsi muscles in Nanhua and Large Yorkshire pigs. In this study, we found that *AK1* expression was significantly higher in the chest muscles compared to the leg muscles, which is consistent with the higher IMP content in the former. Furthermore, AK1 was functionally annotated to ADP deposition and metabolism, and thus regulates IMP deposition directly or indirectly via purine metabolism.

Pyruvate kinase (**PK**) catalyzes the conversion of phosphoenolpyruvate to pyruvate and ATP in the glycolytic pathway. The PKM2 protein can be inactivated via interaction with tyrosine phosphorylated proteins, or by post-translational modifications such as phosphorylation, acetylation, and oxidation (Gui et al., 2013). Apart from its role in cancer (Iqbal et al., 2014), PKM2 is also a causative factor of PSE meat (Nath and Mukherjee, 2014). In addition, the differential expression levels of *PKM2* in the psoas major and semitendinosus muscles affect the quality of the meat. Fu et al. (2013) also observed a positive correlation between *PKM2* expression and chicken quality. Therefore, we hypothesize that PGM2 can regulate the deposition of IMP in muscle via both sugar and purine metabolism pathways.

Glucose phosphate mutase 1 or Phosphoglucomutase 1 (**PGM1**) reversibly catalyzes the transfer of phosphate groups between the first and sixth positions of glucose phosphate and regulates glucose metabolism. Aberrantly high levels of PGM1 not only impair muscle development but are also related to the malignant transformation of cells (Bae et al., 2014). PGM1 deficiency, on the other hand, leads to congenital glycosylation disorder (CDG) and glycogen storage disease, which manifest as short stature, mainly due to PGM1's high expression in skeletal muscles, particularly, the longissimus dorsi, and its relatively low expression in other

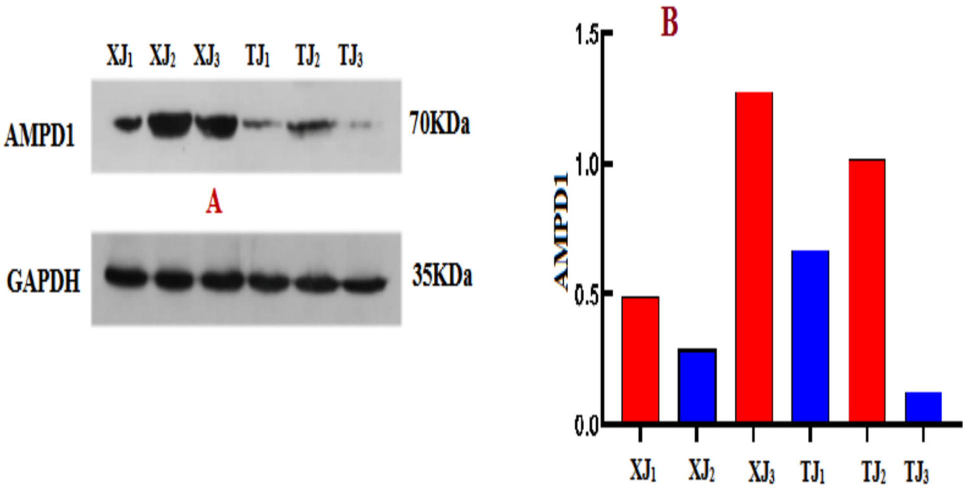

Figure 9. Quantitative verification of the expression of differential protein AMPD1 in the breast and leg muscles of Jingyuan chicken. (A) Western Blot quantitative results; (B) AMPD1 protein gray value calculation results; "XJ" stands for breast muscles, and "TJ" stands for leg muscles.

tissues (Schrapers et al., 2016). In terms of meat quality, PGM1 was negatively correlated with the pH and positively with the L* value and drip loss of beef. In addition, PGM1 is also related to fat deposition (Żelechowska et al., 2012) and boar taint. We detected significant differences in *PGM1* expression levels across the muscle groups of the Jingyuan chicken, and functional annotation showed that PGM1 plays a regulatory role in spatial IMP deposition, although the mechanism remains to be elucidated. Thus, PGM1 not only regulates growth and development but also the muscle quality of livestock and poultry.

CONCLUSIONS

We used the proteomics approach to identify key proteins regulating IMP deposition in different muscle groups of the Jingyuan chicken. A total of 322 differentially expressed proteins were identified in the breast and leg muscles of the Jingyuan chicken, of which AMPD1, AK1, PKM2, PGM1, and PurH/ATIC regulate IMP deposition. Our findings provide a scientific basis for generating high-quality livestock and poultry breeds through gene-editing technology, as well as regulating IMP deposition in the muscle to control flavor. Our study can be applied to the preservation and innovation of the germplasm resources of local broiler breeds, in order to produce safe and high-quality meat.

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Authors’ contributions: ZWH and JZ designed the study, performed the statistical analyses, and drafted the manuscript. ZYC,DWW and XFF wrote the manuscript and analyzed the data. YLG and CYY revised the manuscripts. All authors were involved in interpreting and discussing the results and have read and approved the final manuscript.

DISCLOSURES

The authors declare that they have no competing interests.

SUPPLEMENTARY MATERIALS

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