Proteomics Analysis and Identification of Critical Proteins and Network Interactions that Regulate the Specific Deposition of IMF of Jingyuan Chicken

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Abstract

Background: Improving broiler production efficiency and delivering good quality chicken has become an exciting area of research. Many factors affect the quality of chicken, and the IMF content is one of the critical factors determining the quality of chicken. At present, there are many reports on the molecular mechanism of IMF-specific deposition in chicken; however, only a few reports discuss the specific deposition of IMF in different parts of a chicken.

Methods: In order to analyze the molecular mechanism of IMF specific deposition in different parts of chickens, the present study has selected 180-days old Jingyuan chicken breast and leg muscles as the research materials, using proteomics technology and screening of PRM protein quantitative detection methods, identification and quantitative verification of proteins that control the IMF-specific deposition in the leg muscles and breast muscles of Jingyuan chickens. The protein was analyzed by advanced bioinformatics using GO, KEGG, R language, Gallus_gallus_UniPort, and other biological software, including tools and related databases for screening and identification.

Results: By screening and identifying LC-MS/MS mass spectrometry data and comparing it with the Gallus_gallus_UniPort database, a total of 1940.0 proteins were screened and identified, of which 1317.0 proteins were quantified. Compared with the breast muscles, there are 190 differentially expressed proteins between leg and breast muscles. In the leg muscles, 121 proteins are up-regulated, whereas 69 are down-regulated. Gene Ontology (GO) function annotation and enrichment analysis were done for 190 differential proteins. These proteins participate in a biological process (BP), cellular component (CC), and molecular function (MF) pathways. However, a total of 286 signaling pathways involved 190 differential proteins using a KEGG pathway. Significantly different proteins between the leg and breast muscles were primarily enriched with gga00500Starchandsucrosemetabolism, gga00071 Fattyaciddegradation, gga01212Fattyacidmetabolism, gga03320PPAR signaling pathway. PRM was used to confirm and identify the differential proteins by screening quantitatively; 10 of them are related to fat deposition, namely E1BTT4, E1C0Q5 (ACAT1), F1NC38 (ACADL), Q5F420 (ACSL1), A0A1D5P3S9 (ECI1), Q5ZL56 (ACADS), F1NUQ3 (FABP3), A0A1D5PL36 (ACAA2), F1NR44 (ECHS1), Q5ZIR7 (FABP5) using STRING and Cytoscape software to analyze the network interactions of 10 differentially expressed proteins, resulting in 19 network interaction paths.

Conclusion: We used modern omics technology, omics software, and related databases to screen and identify 3 key signal pathways, including 10 key functional proteins that control IMF-specific deposition in different parts of chickens. At the same time, the present results can be used for different parts of the chicken. Specifically, the molecular mechanism of IMF-specific deposition has been explained, and a key regulatory role is played by the network interaction analysis of proteins that control IMF-specific deposition in different parts of chickens. The results have a scientific value and theoretical significance for improving chicken quality and molecular breeding of high-quality local chicken breeds.

Background

Poultry meat has become the second-largest meat consumption in China; its demand is increasing year by year, showing that poultry meat has become an essential item to human beings (Deng et al. 2016). The growth in poultry production has become excellent due to the contribution made by researchers using improved genetic techniques on the growth rate, feed remuneration, and muscle yield of poultry (Laura et al., 2017; Zhi et al., 2018). Unfortunately, the quality of meat has continued to decline due to poultry production and the steady growth in muscle production (Muazzez et al., 2016). Therefore, improving both the quality and quantity of broilers has become a hot topic in recent research. In intramuscular fat (IMF), fat is distributed among muscle fibers in muscle tissues, whereas IMF content plays a vital role in terms of flavor, freshness, juiciness, taste, tenderness, and color of meat (MA et al. 2015). Studies have shown that IMF is derived from adipocytes between the myofascial membrane and myointimal cell; however, these fat cells are derived from myogenic PDGFR α and progenitor cells (Guan et al. 2017; Wang et al. 2017), including MyoD-directed differentiation of Myf5- and Pax3-non-myogenic progenitor cells (Liu et al. 2012).

Previous studies have suggested that the IMF content can be determined using both chronologically (Lin et al. 2015) and heritability ($h^2 = 0.5$) (Liu et al. 2017). Studies on the molecular mechanism of chicken IMF specific deposition are exhibited into four aspects, which are as follows:

- (i) Researchers have analyzed the biochemical mechanism of IMF affecting meat flavor (Liu et al., 2017).
- (ii) Considering the dimension of development, the deposition rules of IMF have been explored in vivo in which the primary functional genes and signal pathways are regulated at various stages. For example, the late embryonic stage is also one of the critical periods for intramuscular fat deposition. Previous studies have identified the essential roles of PPAR, fatty acid oxidation, cell connectivity, and other related pathways in intramuscular fat deposition (Zhang et al., 2017).
- (iii) The molecular regulatory mechanism for differential fat deposition in breasts, leg muscle, and abdomen was approximately compared with each other by fat deposition on different parts of the body (Cui et al., 2018).
- (iv) It is confirmed that genomic breeding methods have a certain potential in improving intramuscular fat deposition and meat quality (Jiang et al., 2017; Cui et al., 2018; Liu et al., 2019).

In general, the regulation of IMF is the product of the interaction between multiple signaling molecules. Current research on IMF deposition remains primarily at the genetic level and the difference between varieties. However, the regulation of IMF specific deposition for critical proteins in different parts are not available as few related studies on molecular mechanisms and interactions between proteins have so far been obtained.

China has a high resource of high-quality local broiler germplasm, including delicious meat, high tolerance of disease, and strong adaptability. Jingyuan chicken is one of the five district-level livestock and poultry genetic resources in the Ningxia Autonomous Region. It is also a unique broiler breed in the

Animal and Poultry Genetics. We have found that the IMF content of Jingyuan chicken is higher than that of other breeds. However, the specific deposition of IMF in different parts of Jingyuan chicken is not clear. Furthermore, the particular expression and regulation of proteins in its body and the network of interactions need to be deeply analyzed.

Based on the previous research, we have selected a high-quality local broiler breed-Jingyuan chicken, as an objective of the present study. We used the label-free proteomics technique and parallel reaction monitoring method (PRM) to screen and identify the critical functional proteins that regulate the specific deposition IMF on the leg and breast muscles of Jingyuan chicken. The results have shown significant biological and economic value to improve the flavor and quality of chicken and have typical representative significance to the molecular breeding research of high-quality local broilers.

Methods

Reagent

Sequencing grade modified trypsin has been obtained from Promega (Fitchburg, WI). Iodoacetamide (IAA), DTT, TFA, EDTA, urea, tetraethylammonium borohydride (TEAB), 2-DQuantkit, etc., were all been purchased from Sigma (St. Louis, MO) and GE Healthcare (Buckinghamshire, United Kingdom). Besides, protease inhibitor cocktail III, TMT kit, ACN, purified water, formic acid (FA), etc., were procured from Thermo Fisher Scientific (Waltham, MA) and German Fluke (Buches, Germany) companies.

Animals and samples

In this research, 30 (hens) of 180-days old white-feathered Jingyuan chicken with an average live weight of 2.5 ± 0.23 kg were selected from the Chaona chicken breeding center in Pengyang County of Ningxia Hui Autonomous Region. The visible fat, tendon, surface membrane of leg muscle, and pectoralis muscle in a sterile environment were removed and quickly placed at -80°C . We burned and buried animal carcasses after experiments to prevent them from causing environmental waste and damage to human health, following the "Environmental Protection Law of the People's Republic of China" and the "Animal Epidemic Prevention Law of the People's Republic of China,"

Protein extraction and enzymatic hydrolysis

The sample was removed from the refrigerator (-80°C) and ground thoroughly in a mortar containing liquid nitrogen. After grinding into powder, four times the volume of lysis buffer (8M urea, 1% protease inhibitor) was added for ultrasonic lysis. 12000 g of the sample was centrifuged at 4°C for 10 min, removed the cell debris, and the BCA kit determined the protein concentration. Upon dilution of the lysed proteins by dithiothreitol and iodoacetamide, trypsin was added at a mass ratio of 1:50 (trypsin: protein), and enzymatic hydrolysis was performed overnight at 37°C . Trypsin was then added at a 1:100 mass ratio (trypsin: protein) to continue enzymatic hydrolysis for 4 h.

Liquid chromatography-mass spectrometry analysis

The peptide was dissolved in an aqueous solution containing 0.1% formic acid and acetonitrile solution containing 0.1% formic acid using liquid chromatography mobile phase A and mobile phase B, respectively. Then peptide was separated by Nano ultra-high performance liquid system and injected into a capillary ion source for ionization and then analyzed by time to pro-mass spectrometry.

Screening and principal component analysis of differential proteins

The signal abundance of each protein was analyzed using mass spectrometry, and the LFQ intensity value of that protein was obtained using a non-standard quantitative calculation method. The estimation of each sample's relative quantitative value was done based on the protein LFQ intensity value between different samples. The principle of calculation for differential protein expression is to screen for differential proteins with a p-value of < 0.05 . When the differential protein expression shifts by more than 2, it indicates that it is up-regulated during life-long activities. When the differential protein expression varies less than $1/2$, the protein is down-regulated during life activities.

Subcellular Localization

The cells of eukaryotic organisms are specifically subdivided into functionally distinct membrane-bound compartments. Some of the major constituents of eukaryotic cells are extracellular space, cytoplasm, nucleus, mitochondria, Golgi apparatus, endoplasmic reticulum (ER), peroxisome, vacuoles, cytoskeleton, nucleoplasm, nucleolus, nuclear matrix, and ribosomes. We used WOLF SORT, a subcellular localization prediction soft, to predict subcellular localization. However, WOLF SORT is an updated version of PSORT/PSORT II to predict eukaryotic sequences.

Database search and bioinformatics analysis

First, MaxQuant (v1.6.6.0) was used to retrieve the mass spectrometry data, and consequently, Gallus_gallus_UniPort (29475 proteins) database was used. Some parameters in the database were modified and set, including the enzyme restriction method Trypsin/P to eliminate the contaminated proteins on the identification results and reduce the false positive rate (FDR). The number of missing bits was set to 2, whereas Firstsearch obtained the first order precursor-ion mass error tolerance in which the main search was set as 70 ppm. The mass error tolerance of secondary fragment ions was set as 0.04 da. The fixed modification was set for cysteine alkylation, whereas variable modification was established for methionine oxidation, N-terminal acetylation, and deamidation of protein. The FDR for protein identification and PSM identification was set as 1%. The potential connections and differences in their specific functions (GO, KEGG pathways, protein domains, etc.) were analyzed for biological information based on the functional enrichment and clustering specificity of various groups with differentially expressed proteins (or differentially expressed proteins with varying multiples of differential),

Parallel reaction monitoring (PRM-MS) analysis

Specific proteins related to breasts and leg muscles were quantified in LC-PRM/MS by PRM. The results obtained from PRM were calibrated using Xcalibur™ software. The peptides were separated by an ULTRA

high-performance liquid system, injected into an NSI ion source for ionization, and then analyzed by Q ExactiveTM Plus mass spectrometry.

Enrichment-based Clustering

In order to perform further hierarchical clustering based on differentially expressed protein functional classification, such as GO, Domain, Pathway, Complex) we first collated all the categories obtained after enrichment and their P values, then filtered for those enriched in at least one of the clusters with P-value <0.05. This filtered P-value matrix was transformed by the function $x = -\log_{10}(P\text{-value})$. These x values have finally been z-transformed for each functional group. These z scores were then clustered by one-way hierarchical clustering (Euclidean distance, average linkage clustering) in Genesis. Cluster membership was visualized by a heat map using the “heatmap.2” function from “gplots” R-package.

Protein-protein Interaction Network

All differentially expressed protein database accession or sequences have been searched for protein-protein interactions in the STRING database version 10.1. Only the interactions between proteins belonging to the searched data set were selected by removing external candidates. STRING defines a metric called “confidence score” to determine interaction confidence; all interactions with a confidence score of ≥ 0.7 (high confidence) have been reported. STRING interaction network was visualized in R package “networkD3”.

Results

Mass spectrometry data analysis of proteome in Jingyuan chicken Leg muscle and Breast muscle

Label-free quantitative proteomics was used to analyze the Jingyuan chicken leg muscle and breast muscle tissues. 265,391.0 secondary protein spectrograms were identified by LC-MS/MS mass spectrometry, and 85811.0 effective spectrograms were obtained after data search, with a rate of 32.3%. After spectrum analysis, 15,139.0 peptides were identified, among which 12,819.0 peptides were obtained. Finally, 1940.0 proteins were identified, among which 1317.0 proteins could be quantified (Figure 1A). The results showed that the sequence length of detected peptides was mainly distributed between 8 and 32 (Figure 1B). The error between the actual and theoretical values of the identified protein-peptide relative molecular weight was concentrated around 0, less than 20 ppm (Figure 1C). The analysis found that most peptides were less than 200 kDa, and these proteins covered 80% of the identified protein sequences (Figure 1D). The above data indicated that protein identification results are relatively ideal, and the subsequent data analysis could be continued.

Screening and principal component analysis of differential proteins in leg and Breast muscles of Jingyuan chicken

Based on the principle of protein differential expression screening, 190 differentially expressed proteins were found in the leg and breasts muscles. Compared to the breast muscles, 121 proteins in the leg muscles were up-regulated, whereas 69 proteins were downregulated (Figure 2A, B). The principal component analysis was carried out on 190 differential proteins identified in Jingyuan chicken leg muscle and breast muscle, as shown in Figure 2C. The variance contribution rate for the first principal component, PC1, was 53.8%, and the variance contribution rate for the second principal component, PC2, was 17.4%. The contribution rate of PC1 and PC2 reached 71.2%, reflecting most of the information of differential proteins, whereas the proteome of the two parts was significantly different.

Functional classification and GO enrichment analysis of differentially expressed proteins in Jingyuan chicken leg muscle and breast muscle

To further study the biological functions performed by differentially expressed proteins in Jingyuan chicken leg and breast muscles, we performed Gene Ontology (GO) function annotation and enrichment analysis of 190 differential proteins. The biological process (BP), cellular component (CC), and molecular function (MF) are three aspects of the differentially expressed proteins that are discussed. Figure 3 shows that differential proteins are mainly involved in cellular processes (116 proteins, 22%), single-organism process (112 proteins, 21%), metabolic processes (93 proteins, 18%) , biological regulation (47 proteins, reflecting 9%), cellular component organization or biogenesis (33 proteins, representing 6%) and other biological processes. In CCs, differential proteins are involved in the cell (114 differential proteins, accounting for 30%), organelle (87 proteins, reflecting 23%), macromolecular complex (51 proteins, accounting for 14%), membrane (49 proteins, accounting for 13%), extractor region (38 proteins, confirming 10%) and other cell components. In MFs, differential proteins participate in binding (123 proteins, representing 45%), catalytical activity (104 proteins, reflecting 38%), transportation activity (16 proteins, confirming 6%), structural molecular activity (11 proteins, representing 4%) and other molecular functions.

Further, the subcellular localization of 190 differentially expressed proteins has been found in the leg and breast muscles of Jingyuan chicken. The differential proteins were primarily located in the cytoplasm (92 proteins, 48.42%), mitochondria (28 proteins, 14.74%), nucleus (24 proteins, 12.63%), extracellular (21 protein, 11.05%), cytoplasm nucleus (12 proteins, 6.32%), plasma membrane (9 proteins, 4.74%), other (4 proteins, 2.11%) organelles, as shown in Fig. 4. For systematically analyzing the differential protein functions identified in Jingyuan chicken leg and breast muscles, COG (Cluster of Orthologous Groups of proteins) was used to perform gene function annotation and genome evolution analysis on 190 differential proteins. Figure 5 shows the results obtained from this study. The histogram shows participation in energy production and conversion (15 proteins), carbohydrate transportation and metabolism (15 proteins), lipid transportation and metabolism (13 proteins), cytoskeleton (12 proteins), general function prediction (9 proteins), translation, ribosomal structure and biogenesis (5 proteins), intracellular trafficking, secretion and vesicular transport (5 proteins), etc. Biological processes with the highest protein frequency are responsible for energy production and conversion (15 proteins), carbohydrate transport and metabolism (15 proteins), lipid transport, and metabolism (13 proteins). The

biological processes with the lowest frequency of occurrence mainly include post-translational modification, protein turnover, chaperones, coenzyme transport and metabolism, Inorganic ion transport and metabolism, secondary metabolites biosynthesis, transport, and catalyst, etc. However, in the annotation of gene function and genome evolution analysis, 90 different protein functions have not been determined.

KEGG pathway analysis of differentially expressed protein in Jingyuan chicken leg muscle and breast muscle

Different types of proteins perform their biological functions through interaction; however, a single protein cannot independently perform its biological functions as all these proteins are connected through signal pathways. Therefore, the analysis of different protein pathways can help us understand the biological functions more systematically and comprehensively. The regulatory mechanism of protein-protein interaction involves a change of character or occurrence of diseases. The KEGG analysis method may well classify proteins, identify the metabolic or signaling pathways in which these proteins may participate, and obtain information about the proteins involved in a series of biological processes from the cell surface to the nucleus, ultimately helping us to understand the entire organism. KEGG pathway enrichment analysis of 190 proteins differentially expressed in Jingyuan chicken leg and breast muscles showed that 190 differential proteins involved a total of 286 signaling pathways. These differential proteins are involved in more than 20 significant pathways: gga01100 Metabolic pathways, gga00500 Starch, and sucrose metabolism. Figure 6 depicts various signaling pathways: gga00010 Glycolysis/Gluconeogenesis, gga00071 Fatty acid degradation, gga01212 Fatty acid metabolism, gga01200 Carbon metabolism, gga00280 Valine, leucine, and isoleucine degradation, gga03320 PPAR. Thus, proteins with a significant difference between Jingyuan chicken leg and breast muscles are enriched with gga000500 starch and sucrose metabolism, gga00071 Fatty acid degradation, gga01212 fatty acid metabolism, gga03320 PPAR signaling pathway, etc.

Parallel reaction monitoring (PRM) validation of the differential expression protein between leg muscle and breast muscle of Jingyuan chicken

Based on differential protein peptides combined with GO analysis and KEGG pathway analysis, 23 target proteins were selected for PRM quantification. Among them, only 21 have been quantified due to the characteristics of some proteins and the abundance of their expression 10 out of these 21 quantified proteins have been associated with fat deposition, namely E1BTT4, E1C0Q5 (ACAT1). Other 10 proteins are associated with F1NC38 (ACADL), Q5F420 (ACSL1), A0A1D5P3S9 (ECI1), Q5ZL56 (ACADS), F1NUQ3 (FABP3), A0A1D5PL36 (ACAA2), F1NR44 (ECHS1), Q5ZIR7 (FABP5). These proteins are enriched with "gga00071Fattyaciddegradation, gga01212Fattyacidmetabolis, gga03320PPARsignalingpathway" network pathways that regulate fat deposition, as shown in Table 1. The activity of related proteins that regulate fat deposition in Jingyuan chicken leg muscles was found to be significantly more potent than that of breast muscles, further explaining the critical reason why the IMF content of leg muscles was higher than that of breast muscles.

Network Interaction Analysis of IMF-specific Deposited Key Proteins in Jingyuan Chicken Leg and breast Muscles

Protein is the primary executor of life activities, and the interaction between proteins plays a vital role in regulating life activities. In this study, both STRING and Cytoscape software have used to analyze the network interactions of IMF-specific deposition proteins that controlled the leg and breast muscles of Jingyuan chicken to obtain a protein-protein interaction network map. Figure 7 depicts that 8 proteins are involved in the protein-protein interaction network, and they form 19 network interaction paths, indicating that IMF deposition is a very complex and ordered biological process.

Discussion

Meat quality is a comprehensive economic character, including flavor, texture, nutrition, safety, and so on. Many factors affect the quality of domestic chicken, including muscle pH, temperature, the water activity of a reaction system, the interaction of flavor, and the composition of a meat matrix. The factors of production management primarily include breed, age, sex, dietary factors, feeding environment, feeding system, slaughter process, conditions for carcass storage, cooking methods, influencing different degrees of a taste of poultry meat (Wang et al., 2004; Guang et al. 2006; Lu et al. 2007). IMF has a specific relationship with muscle tenderness, juiciness, flavor, and shear strength that can significantly boost the quality of meat (Fernandez et al. 1999; Jeremiah et al. 2003; Hambrecht et al. 2005; Qiankun et al. 2010; Zhang et al. 2018; Wang 2019). It is reflected in two aspects: on the one hand, fat content in muscle is positively correlated with muscle tenderness (Okeudo N & Moss B 2005; Chartrin et al. 2006) due to adipose and connective tissues in the muscle at an intersection state. This morphological structure may loosen the connective tissue by decreasing the physical strength, resulting in a separation of muscle fiber bundles and improving muscle tenderness. Simultaneously, oxidation has an effect of dissolving muscle fiber bundles and may also enhance muscle tenderness and juiciness (Blanchard et al., 2000; Fortin et al., 2005). In contrast, intramuscular fat is rich in phospholipids (approximately 0.5%-1% of fresh muscle weight). Phospholipids contain a large amount of polyunsaturated fatty acids, mostly long-chain polyunsaturated fatty acids. Thus, it can generate aroma substances through the Maillard reaction (JO & AM 1979; Ghasemi H et al. 2016), thereby increasing the meat's flavor. Hence, the deposition of IMF content in muscle plays a crucial role in improving the flavor and quality of muscle.

Genetic and nutritional factors regulate IMF deposition, whereas the present study has a medium genetic factor ($h^2 = 0.5$). Differences in animal species, sex, age, tissue location, etc., cause discrepancies in IMF deposition (Li et al. 2004; Jiang 2010). Previous studies have found that the IMF content in leg muscles is 3-4 times higher than in breast muscles (Li 2008), showing variations in time and tissue of fat deposition in different tissue sections. The mechanism may be related not only to lipid synthesis and degradation under the influence of hormones and enzymes in different parts of adipose tissue but also to a certain degree of variation in the development of microvascular tissue (Leclercq et al., 1984). The high-intensity breeding selection causes a growth speed in broilers, almost reaching its physiological limit, which leads to a significant reduction in the quality of meat.

Besides, 10 differentially expressed proteins were screened and identified to regulate the IMF deposition in the body via direct or indirect pathways. For example, Acetyl CoA Acyltransferase2 (ACAA2) is a key enzyme for fatty acid oxidation. It primarily controls the intermuscular fat by regulating fatty acid metabolism (Sodhi et al. 2014, Miltiadou et al., 2017). Its corresponding target gene is also considered a candidate gene for detecting intermuscular fat content (Miltiadou et al., 2017). Studies have also shown that the expression of the ACAA2 gene in chicken leg muscles is significantly higher than that of pectoral muscles (Liu 2009).

Long-chain acyl-coenzyme A dehydrogenase (ACADL), a coenzyme of acetylase in the body, plays a crucial role in metabolic regulations of sugars, proteins, and lipids, especially in the β oxidation of long-chain fatty acids. Its regulatory mechanism primarily involves control of expression and activity and has a very close relationship with fat metabolism (Luan et al., 2003; Li 2012; Lu et al. 2013). Acyl CoA dehydrogenase short-chain (ACADS) is a non-acyl dehydrogenase responsible for β -oxidation of C4-C6 short-chain fatty acids is also a rate-limiting enzyme for fatty acids degradation (Kruger et al. 2012). ACADS regulates fat deposition by regulating the fatty acid degradation pathway. (Chen & Su, 2015; Vockley, 2008; Peng et al., 2013).

Long-chain fatty acyl CoA synthetase (ACSL) is a critical enzyme in the activation and catalysis of fatty acids (FA), converting long-chain fatty acids into fatty acyl CoA. Studies have shown that the ACSL1 gene encodes a synthetase related to lipid metabolism, expressed in adipose tissue and liver intima (Wang et al. 2016). ACSL1 participates in the synthesis of triglycerides by fatty acyl CoA, promotes the deposition of Fas (Pan et al. 2010), activates FA, and enters the β -oxidation pathway (Ellis et al. 2010). The ACSL1 gene has been reported to affect the fatty acid composition by regulating the total fat content of skeletal muscle (Young et al. 2018).

Heart fatty acid-binding protein (FABP3 or H-FABP) is a member of the fatty acid-binding protein family, expressed in the myocardium and skeletal muscle (Veerkamp & Maatman 1995). It is involved in maintaining an energy balance of the heart and other tissues, regulating muscle fat content, and adipose tissue development (Binas et al. 1999; Shearer et al. 2005; Li et al. 2007). However, overexpression of the FABP3 gene in 3T3-L1 preadipocytes can promote adipogenesis (Yi et al., 2014). The results showed that the FABP3 gene mutation and mRNA expression level had a significant impact on the IMF (Li et al. 2010; Cho et al. 2011), which, in turn, influences muscle tenderness. For example, the FABP3 gene mutation impacts the IMF of pigs (Wang et al. 2015) and goats (Sweeney et al. 2015). Thus, researchers have considered the FABP3 gene as a candidate to regulate the IMF and tenderness of domestic animal muscle. The effect of FABP3 on IMF and meat quality is mainly due to the combination of fatty acids to preserve the concentration gradient of intracellular and extracellular fatty acids, promote fatty acids to enter the cells to increase IMF content (Atay et al. 2019; Shang et al. 2019) and to enhance muscle tenderness (Li et al. 2004; Zhou et al. 2005).

Fatty acid-binding protein 5 (FABP5) is considered an epidermal fatty acid-binding protein (E-FABP), showing that FABP5 regulates lipid metabolism by regulating intracellular fatty acid transport (Dallaglio

et al. 2013). Besides, FABP5 also plays a vital role in regulating cell proliferation, metabolism, and immune response (Yu et al., 2016). Researchers have shown that FABP5 can also regulate the fat traits of livestock and poultry by combining them with long-chain fatty acids to activate the PPARR pathway (Yu et al., 2012). Previous studies showed that FABP5 might be a candidate gene for fat traits and production in livestock and poultry (Estellé et al. al. 2006; Yu 2007; Ojeda et al. 2008; Zhang et al. 2012), explaining that these proteins play a crucial role in fat deposition in livestock and poultry, and their target genes have some expression differences in leg and chest muscles. The present results are consistent with those of previous results, proving the scientificity and importance.

Conclusion

In summary, we have used proteomics technology and PRM protein quantitative detection methods to carry out some basic research on Jingyuan chicken leg muscles and breast muscles. The key proteins obtained by screening provide important molecular materials for further research to increase the IMF content of broilers, leading to new understanding and insights into the molecular mechanism of meat quality differences in different parts of chickens. This research has a biological significance and scientific value for the molecular breeding of high-quality local broilers.

Abbreviations

IMF: Intramuscular fat;

PRM: Parallel reaction monitoring method;

GO: Gene Ontology;

BP: Biological process ;

CC: Cellular component ;

MF: Molecular function;

COG: Cluster of Orthologous Groups of proteins

Declarations

Ethics approval and consent to participate

The experiment was performed according to the animal care and following the guidelines of the Animal Care Committee at Ningxia University. Postoperative animals according to animal harmless treatment methods and principles, scientific treatment.

Consent to publish

All authors agree to publish this paper.

Availability of data and materials

All the data and materials in this paper are true and reliable, with a certain scientific and feasible, fully meet the requirements and needs of experimental design.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

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

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References

Atay E, Guzel M, Amanvermez R, Demir MT, Erenler AK, Ozgen E & Yucel M (2019): Role of Gal-3 and H-FABP in the early diagnosis of acute coronary syndrome. Bratisl Lek Listy 120: 124-130.

Binas B, Danneberg H, Mcwhir J, Mullins L & Clark AJ (1999): Requirement for the heart-type fatty acid binding protein in cardiac fatty acid utilization. The FASEB Journal 13 .

Blanchard PJ, Willis MB, Warkup CC & Ellis M 2000: The influence of carcass backfat and intramuscular fat level on pork eating quality. *J Sci Food Agric* 80 .

Chartrin P, Méteau K, Juin H, Bernadet M D, Guy G, Larzul C, Rémignon H, Mourot J, Duclos M J & Baéza E 2006: Effects of intramuscular fat levels on sensory characteristics of duck breast meat. *Poult Sci* 85 .

Chen Y & Su Z 2015: Reveal genes functionally associated with ACADS by a network study. *Gene* 569 .

Cho KH, Kim MJ, Jeon GJ & Chung HY 2011: Association of genetic variants for FABP3 gene with back fat thickness and intramuscular fat content in pig. *Mol Biol Rep* 38 .

Cui H, Zheng M, Zhao G, Liu R & Wen J 2018: Identification of differentially expressed genes and pathways for intramuscular fat metabolism between breast and thigh tissues of chickens. *BMC Genomics* 19: 55.

Dallaglio K, Marconi A, Truzzi F, Lotti R, Palazzo E, Petrachi T, Saltari A, Coppini M & Pincelli C 2013: E-FABP induces differentiation in normal human keratinocytes and modulates the differentiation process in psoriatic keratinocytes in vitro. *Exp Dermatol* 22 .

Ellis JM, Li LO, Wu PC, Koves TR, Ilkayeva O, Stevens RD, Watkins SM, Muoio DM & Coleman RA 2010: Adipose acyl-CoA synthetase-1 directs fatty acids toward beta-oxidation and is required for cold thermogenesis. *Cell Metab* 12: 53-64.

Estellé J, Pérez-Enciso M, Mercadé A, Varona L, Alves E, Sánchez A & Folch JM 2006: Characterization of the porcine FABP5 gene and its association with the FAT1 QTL in an Iberian by Landrace cross. *Anim Genet* 37 .

Fan J, Shan C, Kang H, Elf S, Xie J, Tucker M, Gu T, Aguiar M, Lonning S, Chen H, Mohammadi M, Britton LP, Garcia BA, Alečković M, Kang Y, Kaluz S, Devi N, Van Meir EG, Hitosugi T, Seo JH & Sagar. 2014: Tyr Phosphorylation of PDP1 Toggles Recruitment between ACAT1 and SIRT3 to Regulate the Pyruvate Dehydrogenase Complex. *Mol Cell* 53 .

Fernandez X, Monin G, Talmant A, Mourot J & Lebret B 1999: Influence of intramuscular fat content on the quality of pig meat - 1. Composition of the lipid fraction and sensory characteristics of m. longissimus lumborum. *Pubmed* 53 .

Fortin A, Robertson W M & Tong A K W 2005: The eating quality of Canadian pork and its relationship with intramuscular fat. *Meat Sci* 69 .

Fumiyo, Kasuya, Ryota, Nishizawa, Teiichi, Masuyama, Maya & Kazumi 2010: Evaluation of a screening method by liquid chromatography-tandem mass spectrometry for estimating effect of drugs on the activation and β -oxidation of fatty acids in mitochondria. *J Pharm Pharmacol* .

- Ghasemi H A, Shivazad M, Mirzapour Rezaei S S & Karimi Torshizi M A 2016: Effect of synbiotic supplementation and dietary fat sources on broiler performance, serum lipids, muscle fatty acid profile and meat quality. *Br Poult Sci* 57 .
- Griffin HD, Butterwith SC & Goddard C 1987: Contribution of lipoprotein lipase to differences in fatness between broiler and layer-strain chickens. *Br Poult Sci* 28: 197-206.
- Guan L, Hu X, Liu L, Xing Y, Zhou Z, Liang X, Yang Q, Jin S, Bao J, Gao H, Du M, Li J & Zhang L 2017: bta-miR-23a involves in adipogenesis of progenitor cells derived from fetal bovine skeletal muscle. *Sci Rep* 7: 43716.
- Gurvitz A, Wabnegger L, Yagi AI, Binder M, Hartig A, Ruis H, Hamilton B, Dawes IW, Hiltunen JK & Rottensteiner H 1999: Function of human mitochondrial 2,4-dienoyl-CoA reductase and rat monofunctional Delta3-Delta2-enoyl-CoA isomerase in beta-oxidation of unsaturated fatty acids. *Biochem J* 344 Pt 3: 903-914.
- Haapalainen AM, Meriläinen G & Wierenga RK 2005: The thiolase superfamily: condensing enzymes with diverse reaction specificities. *Trends Biochem Sci* 31 .
- Hambrecht E, Eissen JJ, Newman DJ, Smits CH, Verstegen MW & den Hartog LA 2005: Preslaughter handling effects on pork quality and glycolytic potential in two muscles differing in fiber type composition. *J Anim Sci* 83: 900-907.
- Hiltunen JK & Qin YM 2000: β -Oxidation - Strategies for the metabolism of a wide variety of acyl-CoA esters. *Biochim Biophys Acta* 1484: 117-128.
- Hwa JS, Park HJ, Jung JH, Kam SC, Park HC, Kim CW, Kang KR, Hyun JS & Chung KH 2005: Identification of proteins differentially expressed in the conventional renal cell carcinoma by proteomic analysis. *J Korean Med Sci* 20: 450-455.
- J.O. I & A. M. P 1979: ROLE OF PHOSPHOLIPIDS AND TRIGLYCERIDES IN WARMED-OVER FLAVOR DEVELOPMENT IN MEAT MODEL SYSTEMS. John Wiley & Sons, Ltd (10.1111) 44 .
- Janssen U, Davis EM, Le Beau MM & Stoffel W 1997: Human mitochondrial enoyl-CoA hydratase gene (ECHS1): structural organization and assignment to chromosome 10q26.2-q26.3. *Genomics* 40: 470-475.
- Jeremiah LE, Dugan ME, Aalhus JL & Gibson LL 2003: Assessment of the relationship between chemical components and palatability of major beef muscles and muscle groups. *Meat Sci* 65: 1013-1019.
- Ji H & Friedman MI 2007: Reduced capacity for fatty acid oxidation in rats with inherited susceptibility to diet-induced obesity. *Metabolism* 56: 1124-1130.

- Jiang M, Fan WL, Xing SY, Wang J, Li P, Liu RR, Li QH, Zheng MQ, Cui HX, Wen J & Zhao GP 2017: Effects of balanced selection for intramuscular fat and abdominal fat percentage and estimates of genetic parameters. *Poult Sci* 96: 282-287.
- Kilponen JM & Hiltunen JK 1993: β -Oxidation of unsaturated fatty acids in humans Isoforms of Δ^3 , Δ^2 -enoyl-CoA isomerase .
- Kruger C, Kumar KG, Mynatt RL, Volaufova J & Richards BK 2012: Brain transcriptional responses to high-fat diet in Acads-deficient mice reveal energy sensing pathways. *PLoS One* 7: e41709.
- Lake SL, Jmor F, Dopierala J, Taktak AFG, Coupland SE & Damato BE 2011: Multiplex Ligation-Dependent Probe Amplification of Conjunctival Melanoma Reveals Common BRAF V600E Gene Mutation and Gene Copy Number Changes. *Invest Ophthalmol Vis Sci* 52 .
- Laura B, Rosa C & Carlos A 2017: Microbial loads and antibiotic resistance patterns of *Staphylococcus aureus* in different types of raw poultry-based meat preparations. *Poult Sci* 96.
- Leclercq B, Hermier D & Salichon M R 1984: Effects of age and diet on plasma lipid and glucose concentrations in genetically lean or fat chickens. *Reproduction, nutrition, developpement* 24 .
- Li A, Zhang Y, Zhao Z, Wang M & Zan L 2016: Molecular Characterization and Transcriptional Regulation Analysis of the Bovine PDHB Gene. *PLoS One* 11: e0157445.
- Li B, Zerby HN & Lee K 2007: Heart fatty acid binding protein is upregulated during porcine adipocyte development. *J Anim Sci* 85: 1651-1659.
- Li Q, Zuo LL, Lin YQ, Xu YO, Zhu JJ, Liao HH, Lin S, Xiong XR & Wang Y 2016: Cloning and Expression of SFRP5 in Tibetan Chicken and its Relationship with IMF Deposition. *Anim Biotechnol* 27: 231-237.
- Li X, Kim S, Choi J, Lee Y, Lee C, Choi B, Kim T, Choi Y, Kim J & Kim K 2010: Investigation of porcine FABP3 and LEPR gene polymorphisms and mRNA expression for variation in intramuscular fat content. *Mol Biol Rep* 37 .
- Liu L, Cui H, Fu R, Zheng M, Liu R, Zhao G & Wen J 2017: The regulation of IMF deposition in breastis major of fast- and slow- growing chickens at hatching. *J Anim Sci Biotechnol* 8: 77.
- Liu R, Wang H, Liu J, Wang J, Zheng M, Tan X, Xing S, Cui H, Li Q, Zhao G & Wen J 2017: Uncovering the embryonic development-related proteome and metabolome signatures in breast muscle and intramuscular fat of fast-and slow-growing chickens. *BMC Genomics* 18: 816.
- Liu R, Zheng M, Wang J, Cui H, Li Q, Liu J, Zhao G & Wen J 2019: Effects of genomic selection for intramuscular fat content in breast muscle in Chinese local chickens. *Anim Genet* 50: 87-91.

- Liu W, Liu Y, Lai X & Kuang S (2012): Intramuscular adipose is derived from a non-Pax3 lineage and required for efficient regeneration of skeletal muscles. *Dev Biol* 361: 27-38.
- Lu Q, Wen J & Zhang H (2007): Effect of chronic heat exposure on fat deposition and meat quality in two genetic types of chicken. *Poult Sci* 86: 1059-1064.
- Ma JS, Chang WH, Liu GH, Zhang S, Zheng AJ, Li Y, Xie Q, Liu ZY & Cai HY (2015): Effects of flavones of sea buckthorn fruits on growth performance, carcass quality, fat deposition and lipometabolism for broilers. *Poult Sci* 94: 2641-2649.
- Miltiadou D, Hager-Theodorides AL, Symeou S, Constantinou C, Psifidi A, Banos G & Tzamaloukas O (2017): Variants in the 3' untranslated region of the ovine acetyl-coenzyme A acyltransferase 2 gene are associated with dairy traits and exhibit differential allelic expression. *J Dairy Sci* 100: 6285-6297.
- Muazzez C, Yılmaz Ş, Figen K, Hakan BÖ & Selim M (2016): Comparison of Carcass Characteristics, Meat Quality, and Blood Parameters of Slow and Fast Grown Female Broiler Chickens Raised in Organic or Conventional Production System. *Asian-Australas J Anim Sci* 29 .
- Ojeda A, Estellé J, Folch JM & Pérez-Enciso M (2008): Nucleotide variability and linkage disequilibrium patterns at the porcine FABP5 gene. *Anim Genet* 39: 468-473.
- Okeudo N J & Moss B W (2005): Interrelationships amongst carcass and meat quality characteristics of sheep. *Meat Sci* 69 .
- Peng Y, Xiang H, Chen C, Zheng R, Chai J, Peng J & Jiang S (2013): MiR-224 impairs adipocyte early differentiation and regulates fatty acid metabolism. *The international journal of biochemistry and cell biology* 45: 1585-1593.
- Qiankun Z, Hong-Gu L, Jung-A H, Bae KE, Kee KS, Jinlong Y, Myunggi B, Yannan S, Sang-Hoon K, Kang-Seok S & Yun-Jaie C (2010): Differentially expressed proteins during fat accumulation in bovine skeletal muscle. *Meat Sci* 86 .
- Shang P, Zhang B, Zhang J, Duan M, Wu L, Gong X, Tang K, Zhang H & Chamba Y (2019): Expression and single-nucleotide polymorphisms of the H-FABP gene in pigs. *Gene* 710: 156-160.
- Shearer J, Fueger PT, Rottman JN, Bracy DP, Binas B & Wasserman DH (2005): Heart-type fatty acid-binding protein reciprocally regulates glucose and fatty acid utilization during exercise. *Am J Physiol Endocrinol Metab* 288: E292-297.
- Sodhi SS, Ghosh M, Song KD, Sharma N, Kim JH, Kim NE, Lee SJ, Kang CW, Oh SJ & Jeong DK (2014): An approach to identify SNPs in the gene encoding acetyl-CoA acetyltransferase-2 (ACAT-2) and their proposed role in metabolic processes in pig. *PLoS One* 9: e102432.

Sweeney T, O'Halloran AM, Hamill RM, Davey GC, Gil M, Southwood OI & Ryan MT 2015: Novel variation in the FABP3 promoter and its association with fatness traits in pigs. *Meat Sci* 100 .

Veerkamp JH & Maatman RG 1995: Cytoplasmic fatty acid-binding proteins: Their structure and genes. *Prog Lipid Res* 34 .

Vockley J 2008: Metabolism as a complex genetic trait, a systems biology approach: Implications for inborn errors of metabolism and clinical diseases. *J Inher Metab Dis* 31 .

Wang B, Fu X, Liang X, Deavila JM, Wang Z, Zhao L, Tian Q, Zhao J, Gomez NA, Trombetta SC, Zhu MJ & Du M 2017: Retinoic acid induces white adipose tissue browning by increasing adipose vascularity and inducing beige adipogenesis of PDGFR α + adipose progenitors. *Cell Discov* 3: 17036.

Wang D, Wang N, Li N & Li H 2009: Identification of differentially expressed proteins in adipose tissue of divergently selected broilers. *Poult Sci* 88 .

Wang L, Li L, Jiang J, Wang Y, Zhong T, Chen Y, Wang Y & Zhang H 2015: Molecular characterization and different expression patterns of the FABP gene family during goat skeletal muscle development. *Mol Biol Rep* 42 .

Wang Y, Chen H, Han D, Chen Y, Muhatai G, Kurban T, Xing J & He J 2017: Correlation of the A-FABP Gene Polymorphism and mRNA Expression with Intramuscular Fat Content in Three-Yellow Chicken and Hetian-Black Chicken. *Anim Biotechnol* 28: 37-43.

Wang Y, Hui X, Wang H, Kurban T, Hang C, Chen Y, Xing J & Wang J 2016: Association of H-FABP gene polymorphisms with intramuscular fat content in Three-yellow chickens and Hetian-black chickens. *J Anim Sci Biotechnol* 7: 9.

Wu C, Wu Y, Liu C, Wang Y, Na W, Wang N & Li H 2016: Comparative proteome analysis of abdominal adipose tissues between fat and lean broilers. *Proteome Sci* 14 .

Yi B, Wang J, Wang S, Yuan D, Sun J, Li Z, Mao Y, Hou Q & Liu W 2014: Overexpression of Banna mini-pig inbred line fatty acid binding protein 3 promotes adipogenesis in 3T3-L1 preadipocytes. *Cell Biol Int* 38: 918-923.

Yokoyama Y, Kuramitsu Y, Takashima M, Iizuka N, Toda T, Terai S, Sakaida I, Oka M, Nakamura K & Okita K 2004: Proteomic profiling of proteins decreased in hepatocellular carcinoma from patients infected with hepatitis C virus. *Proteomics* 4: 2111-2116.

Young PA, Senkal CE, Suchanek AL, Grevenko TJ, Lin DD, Zhao L, Crunk AE, Klett EL, Füllekrug J, Obeid LM & Coleman RA 2018: Long-chain acyl-CoA synthetase 1 interacts with key proteins that activate and direct fatty acids into niche hepatic pathways. *J Biol Chem* 293: 16724-16740.

Yu C, Liang X, Lipsky S, Karaaslan C, Kozakewich H, Hotamisligil GS, Bischoff J & Cataltepe S (2016): Dual role of fatty acid-binding protein 5 on endothelial cell fate: a potential link between lipid metabolism and angiogenic responses. *Angiogenesis* 19 .

Yu S, Levi L, Siegel R & Noy N (2012): Retinoic acid induces neurogenesis by activating both retinoic acid receptors (RARs) and peroxisome proliferator-activated receptor β/δ (PPAR β/δ). *J Biol Chem* 287: 42195-42205.

Yun-feng LU, Ji-bao C, Bo Z, Qing-gang LI, Zhi-xiu W, Hao Z & Ke-liang WU (2017): Cloning, expression, and polymorphism of the ECI1 gene in various pig breeds. *Journal of Integrative Agriculture* 16: 1789-1799.

Zhang M, Yan FB, Li F, Jiang KR, Li DH, Han RL, Li ZJ, Jiang RR, Liu XJ, Kang XT & Sun GR (2017): Genome-wide DNA methylation profiles reveal novel candidate genes associated with meat quality at different age stages in hens. *Sci Rep* 7: 45564.

Zhang Y, Wang Y, Wang X, Ji Y, Cheng S, Wang M, Zhang C, Yu X, Zhao R, Zhang W, Jin J, Li T, Zuo Q & Li B (2018): Acetyl-coenzyme A acyltransferase 2 promote the differentiation of sheep precursor adipocytes into adipocytes. *J Cell Biochem* .

Zhao G P, Chen J L, Zheng M Q, Wen J & Zhang Y (2007): Correlated responses to selection for increased intramuscular fat in a Chinese quality chicken line. *Poult Sci* 86 .

Zhichao X, Yuting L, Guiying W, Changrong G, Guanghong Z, Wangang Z & Guozhou L (2018): ¹H NMR-based water-soluble lower molecule characterization and fatty acid composition of boiled Wuding chicken during processing. *J Sci Food Agric* .

Chen Gangyao, Tang Zhigang, Wen Chao, Yang Weibing and Zhou Yanmin (2012): Effect of oligomeric chitosan on antioxidant performance, lipid metabolism and muscle quality of growing and finishing pigs. *Food and feed industry*: 54-57.

Liu Chen. A preliminary study on the biochemical and molecular mechanism of the tissue differences in chicken muscle fat and inosine content: Chinese Academy of Agricultural Sciences, 2009.

Chen Jilan, Wen Jie, Wang Shubai, Zhao Guiping, Zheng Maiqing and Li Xiaohua (2005): Research on the deposition of chicken inosine and intramuscular fat. *Journal of Animal Science and Veterinary Medicine*: 843-845.

Chen Jilan, Wen Jie, Zhao Guiping, Zheng Maiqing and Yang Ning (2005): Estimation of genetic parameters of meat flavor traits such as chicken inosine and intramuscular fat. *Heredity*: 42-46.

Dou Tengfei, Wang Shanrong, Liu Lixian, Hu Wenyuan, Tong Huiquan, Gu Dahai, Li Qihua, Jia Junjing, Ge Changrong and Xu Zhiqiang (2016): Correlation between GS gene expression and muscle glycogen content and pH in Wuding chicken and Avian broiler Research. *Chinese Poultry* 38: 17-20.

Li Huifang and Chen Kuanwei (2004): Comparison of muscle inosine and fatty acid content in different chicken breeds. *Journal of Yangzhou University*: 9-11+32.

Li Wufeng, Xu Shangzhong, Cao Honghe and Li Hongbin (2004): Correlation analysis of genetic variation and meat quality traits of the second intron of H-FABP gene in three hybrid cattle breeds. *Journal of Animal Science and Veterinary Medicine*: 252-255.

Lin Yaqiu, Xu Yaou, Zhang Runfeng, Zhang Ming, Zuo Bin and Zheng Yucai (2015): Timing expression of Chemerin and ChemR23 genes in Tibetan chicken and their correlation with intramuscular fat content. *Journal of Animal Science and Veterinary Medicine* 46: 1290-1299.

Yue Ying. Research on liver proteomics of broilers with different genotypes: Chinese Academy of Agricultural Sciences, 2013.

Liu Haifeng, Yan Zhenxin, Dong Han and Li Xuwei (2010): PPAR γ , DECR1 and ECHS1 gene expression patterns during differentiation of porcine preadipocytes. *Journal of Sichuan Agricultural University* 28: 215-218.

Liu Rong, Ju Yanfang, Yang Jinju, Du Xuemei, Chen Yong, Liu Ying, Gao Jianen and Sun Qihong (2008): Preparation and identification of monoclonal antibodies to enoyl-CoA hydrolase. *Journal of Cellular and Molecular Immunology*: 387-388+392.

Lu Yunfeng, Li Qinggang, Wang Zhixiu, Zhang Bo, Wu Keliang and Zhang Hao (2015): Polymorphism of ECI1 gene in Huai pig breeds and its correlation with back fat thickness. *Chinese Journal of Animal Science* 51: 1-4 .

Lu Zhaoning, Wang Wei and Yan Jingbin (2013): the regulatory mechanism of long-chain acyl CoA dehydrogenase. *Journal of medical molecular biology* 10: 172-176

Luan Yihong, Lu Ning, Wang Jiwei and Yi Qing (2003): biochemical function and application of coenzyme A. *Chinese Journal of biochemical medicine* 24: 159-161

Pan Zhixiong, Lu Jia, Lu Lizhi and Wang Jiwen (2010): cloning of goose ACSL1 gene and its role in the formation of goose fatty liver. *Journal of animal husbandry and veterinary medicine* 41: 1407-1413

Yu Jiahui. Relationship between E-FABP gene polymorphism and related traits in Jinghai Yellow Chicken: Yangzhou University, 2007

Wang Qianqian, Yang Biao, Xia Lili, Sun Xiaoxian, Geng Tuoyu and Gong Daoqing (2016): Cloning of goose acetyl-CoA acyltransferase 2 gene and its expression changes in the formation of goose fatty liver. *Journal of Animal Husbandry and Veterinary Medicine* 47: 700-708.

Wang Qin (2019): Research progress on factors affecting muscle fat content in poultry and related genes. *Poultry Science*: 53-58.

Li Peng. Characteristics and regulation of liver fatty acid oxidative metabolism in ketosis cows: Jilin University, 2012.

Li Wenjuan. Screening of Chicken Fat Quality Related Genes for Fat Metabolism and Nutritional Regulation: Chinese Academy of Agricultural Sciences, 2008

Jiang Rui. The effect and mechanism of niacin on chicken fat metabolism and meat quality: Chinese Academy of Agricultural Sciences, 2010.

Zhang Guangmin. The effect of conjugated linoleic acid on the quality of meat and chicken meat and its mechanism of action: Chinese Academy of Agricultural Sciences, 2006.

Wang Shubai. Chicken Inosinate Deposition and Nutritional Regulation: Chinese Academy of Agricultural Sciences, 2004.

Yu Mengmeng, Li Meng, Li Shanshan, et al. Effect of Fatty Acid Binding Protein 5 on Milk Fat Synthesis of Dairy Cow Mammary Epithelial Cells. Chinese Journal of Animal Husbandry and Veterinary Medicine, 2017,44(04):941-949.

Zang Shasha, Liu Yixuan, Song Guangyao and Wang Chao (2014): The effect of Jinlida on skeletal muscle lipid deposition and mitochondrial function in insulin resistant rats. Chinese Patent Medicine 36: 1371-1376.

Zhang Qingqiu, Shi Hui, Wang Yuxiang, Li Hui and Wang Qigui (2012): Effects of changes in A-FABP gene expression in chicken preadipocytes on PPAR γ , perilipin and E-FABP expression. Journal of Animal Science and Veterinary Medicine 43: 1531-1538.

Zhou Guoli, Zhu Qi, Guo Shanli and Wu Yuhou (2005): Analysis of the polymorphism of Luxi cattle H-FABP gene and its relationship with meat quality traits. Northwest Agricultural Journal: 10-12.

Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

Figure 1

The results of label-free proteomics on protein identification of Jingyuan chicken leg and pectoral muscle tissues; A: Basic statistics of mass spectrometry data results; B: Distribution of peptide sequence length after digestion of the identified proteins; C: The molecular mass error distribution diagram of the identified protein peptides; D: The mass distribution diagram of the identified protein molecules. Note: TJ stands for leg muscles and XJ stands for pectoral muscles.

Figure 2

Statistical analysis of differentially expressed proteins of Jingyuan chicken leg muscles and pectoral muscles; A: Statistical analysis of differentially expressed proteins of Jingyuan chicken leg muscles and pectoral muscles; B: Quantitative volcano graph of differentially expressed proteins of Jingyuan chicken leg muscles and pectoral muscles; C: Two dimensional scatter chart of protein quantitative main component analysis (PCA) between repeated samples of Jingyuan chicken leg muscle and chest muscle. Note: TJ stands for leg muscles and XJ stands for pectoral muscles.

Figure 3

Statistical distribution of Jingyuan chicken leg muscle and pectoral muscle differentially expressed proteins in GO secondary classification. Note: TJ stands for leg muscles and XJ stands for pectoral muscles.

Figure 4

Location map of differentially expressed proteins of Jingyuan chicken leg muscle and pectoral muscle in subcellular structure. Note: TJ stands for leg muscles and XJ stands for pectoral muscles.

Figure 5

COG function classification and distribution of differentially expressed proteins in Jingyuan chicken leg muscle and pectoral muscle. Note: TJ stands for leg muscles and XJ stands for pectoral muscles.

Figure 6

Enrichment and distribution map of differentially expressed proteins of Jingyuan chicken leg muscle and pectoral muscle in GO functional classification. Note: TJ stands for leg muscles and XJ stands for pectoral muscles.

Figure 7

Network interaction path diagram of differentially expressed proteins of Jingyuan chicken leg muscle and pectoral muscle. Note: TJ stands for leg muscles and XJ stands for pectoral muscles.

Supplementary Files

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