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Proteomics Analysis Suggests Mitochondria Disorders and Cell Death Lead to Spaghetti Meat Myopathy

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Abstract: Spaghetti meat (SM) is a myopathy that affects the structural integrity of *pectoralis major* muscles in broilers and accounts for decreased meat acceptability and significant economic losses for the industry. The causative mechanisms have not been as extensively investigated as other myopathies such as wooden breast. Proteomics analysis allows for the investigation alterations in muscle composition. The aim of this study was to investigate proteins related to mitochondria function in SM using proteomics analysis. Severe SM and normal *pectoralis major* were collected from a broiler processing plant. The analysis identified 1903 proteins. Results suggested that SM exhibited greater cellular stress and cell death as evidenced by increased N-myc downstream-regulated gene 1, plastin 3, ribosomal protein L10, and calnexin (FDR < 0.05 for all). Increased cell stress potentially impacted nonsense meditated decay pathway (eliminates mRNA containing premature translation termination codons). The SM myopathy downregulated several compounds related to mitochondria function and glucose metabolism pathways including basic leucine zipper and w2 domains 2, eukaryotic translation initiation factor 3 subunit B, pyruvate kinase L/R, thioredoxin-dependent peroxide reductase mitochondrial, enolase 3, creatine kinase mitochondrial 2, succinate-CoA ligase GDP/ADP-forming subunit alpha1, calcium voltage-gated channel auxiliary subunit alpha2delta 1, and complement 1q binding protein C (FDR < 0.05 for all), indicating a disruption in energy production and cell health. Current results suggested that SM impaired energy production and mitochondria function and elevated cell death. Although this study provides valuable information regarding possible mechanisms involved in the SM myopathy, further investigations are required to fully understand the mechanisms and their connections to other vital pathways.

Key words: broiler chicken, spaghetti meat, cell death, mitochondrial dysfunction, proteomics analysis
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Introduction

In the last few years, several muscular abnormalities known as wooden breast, white striping, and spaghetti meat (SM) have afflicted *pectoralis major* muscles in broiler chickens and reduced the quality of the final meat products (Kuttappan et al., 2016; Baldi et al., 2021). Of these breast myopathies, SM was the most recently recognized and therefore is the least investigated to date. The SM fillets are soft with separated muscle fiber bundles resulting in stringy, spaghetti-like appearance. Due to their appearance and lack of structural integrity, SM fillets are downgraded and can only be used in further processed products, which leads to significant losses for the industry (Baldi et al., 2021). Unfortunately, the exact cause of the SM myopathy is unknown, making it difficult to develop effective preventative measures. Different nutritional and management strategies have been examined to reduce the incidence of SM and to limit the impact of the abnormality on product quality (Baldi et al., 2021). However, none of the strategies have identified a promising solution to overcome the issue. Therefore, a better understanding of the involved mechanisms is required to make it possible to develop effective strategies to cope with the issue.

Previous research has shown that intensive genetic selection in fast-growing birds could be responsible for alterations to the structure, function, and metabolic characteristics of muscle fibers (Clark and Velleman, 2016; Soglia et al., 2016; Shakeri et al., 2024). Furthermore, a few studies demonstrated that SM incidence was associated with cellular stress, mitochondria dysfunction, and hypoxia in the tissue (Petracci et al., 2019; Baldi et al., 2021). Mitochondria are essential for normal cell function, regulation of reactive oxygen species (ROS), and apoptosis (Wang et al., 2022b). Genetic alterations and environmental factors increase the possibility of mutations of mitochondrial proteins, which can lead to mitochondrial malfunction. Therefore, mitochondrial quality control by proteins involved in various surveillance mechanisms is critical for viability and normal cell function (Wang et al., 2022b).

To better understand muscle alterations and possible pathways involved in the SM myopathy, it is necessary to identify changes that occur in the biochemical composition of afflicted muscle tissue. A novel method for doing this type of investigation is proteomics analysis. The objective of this study was to use a proteomics approach to identify key biochemical differences involved in mitochondria function between normal broiler breast muscle and muscle exhibiting the SM myopathy. Although this study identified several compounds that were altered in SM compared to normal muscle tissues, this paper focused on compounds related to mitochondria function, cellular stress, and related pathways. To this end, we conducted proteomics analyses comparing normal broiler breast muscle with muscle exhibiting the SM myopathy.

Materials and Methods

Experimental design

An animal use proposal was not required for this experiment as 300 pound breast fillet samples (*pectoralis major*) were collected at ~3 h postmortem from the deboning line of a commercial processing facility where birds (8 wk old male Ross broilers) were stunned and slaughtered according to standard industry procedures. Samples were kept cool on ice during transportation (~1 h) to the U.S. National Poultry Research Center (Athens, GA, USA). In total, 8 severe SM and 8 normal (N) breast fillets were selected based on visual and tactile assessment by a team of trained experts. Severe *pectoralis major* muscles showed extensive superficial lacerations on their cranial and/or caudal surface. Muscle fibers separated from each other (Baldi et al., 2021). The numbers of selected birds were based on previous publications (Wagle et al., 2023; Kong et al., 2024). Muscle samples from the cranial end of the fillets were collected, snap frozen in liquid nitrogen, and stored at −80 °C until further analysis.

Proteomics analysis of breast meat

The preparation and analysis of samples for proteomics were performed by a commercial analytical service (Dalton Bioanalylytics Inc., Los Angeles, California). The procedure was divided into 4 phases (homogenization, digestion, extraction, and Liquid chromatography-mass spectrometry LC-MS analysis) as briefly described in a previous report (Kong et al., 2024). A total of 16 samples were analyzed (8 severe SM and 8 N, the samples were replicated as a group). Each sample (5–6 g) was dissected on ice into 30 mg pieces. The samples were then homogenized using a bead mill (4 cycles of 30 s homogenizing then 30 s on ice). Trypsin was added to digest the samples for 2 h at 37 °C. Additional ethanol was used to perform digestion. Then, precipitate was pelleted by centrifugation for 5 min at 14,000 relative centrifugal force (RCF). Clarified supernatant was transferred for analysis. The procedures are summarized in Table 1.

Data processing and analysis

The data were processed by Dalton Bioanalylytics Inc. using MSFragger software for proteins. An ID library was built on pooled samples. Differential abundances between N and SM were calculated using $\log_2$ fold change. Proteins showing $P < 0.05$ in the comparison between N and SM were considered differentially abundant. Multiple testing was addressed using false discovery rate (FDR) correction of nominal

Table 1. LC-MS method used to analysis spaghetti vs. normal meats.

	Steps[1]	Details
1	Microflow mixed mode UHPLC	- 125 min heart-cut 2D-LC - HILIC (0–35 min hydrophilic analytes)
2	RP (CSH C18)	- 35–73 min peptide analytes - 73–100 min lipophilic analytes - 100–125 min re-equilibration
3	Mobile phase additives	Formic acid and ammonium acetate
4	High-resolution MS (Thermo QE+)	- Ions identification by DDA • (±) ddMS2 on metabolite extracts • ddMS2 high pH peptide fractions - Quantification • MS1 BoxCar acquisition (msx-tSIM)

[1]Ions identified by DDA MS/MS. Ions quantified by BoxCar MS. Label free quantification by 5 ppm and 1 min tolerance. IDs matched between runs. Ions were condensed into molecular identities by averaging ions.

significance (*P* value). Differential associations were visualized using volcano plots with dimensionality reduction plots (green dots FDR < 0.05, red dots FDR < 0.2, and black dots FDR > 0.2).

Results and Discussion

A total of 1903 proteins were identified using proteomics analysis in the SM fillets. The data indicated several significant alterations in protein levels that potentially impaired cell health and mitochondria function (Figure 1). All of the compounds in Figure 1 were not discussed in this paper as the focus was on mitochondria function and cell damage. The discussed compounds in this study are listed in Table 2. The data showed that the SM condition altered glucose pathways (encouraging glucose catabolism in cells) and nonsense mediated decay (NMD) pathways. NMD detects and destroys transcripts containing premature termination codons to prevent the production of truncated proteins that could harm cells. Furthermore, NMD downregulates expression of faulty genes (Nickless et al., 2017) and regulates the expression of normal transcripts (Hug et al., 2016). The data showed that several proteins (PLS3, NDRG1, CANX, and RPL10) were upregulated in SM samples whereas several other proteins (BZW2, EIF3B, PKLR, PRDX3, ENO3, CKMT2, SUCLG1, CACNA2D1, and C1QBP) were downregulated (Figure 1 and Table 2). The SM myopathy is associated with a decrease in muscle protein content and alterations to the connective tissue leading to the formation of large intracellular spaces (Baldi et al., 2018). It has been reported that reducing cell death and apoptosis could lead to improved connective tissue structure (Chen et al., 2023). Furthermore, as mitochondria are responsible for generating ATP, abnormalities in the mitochondria have been shown to play an essential role in cell death (Waterhouse, 2003). It is worth mentioning that there are similarities between woody breast and SM

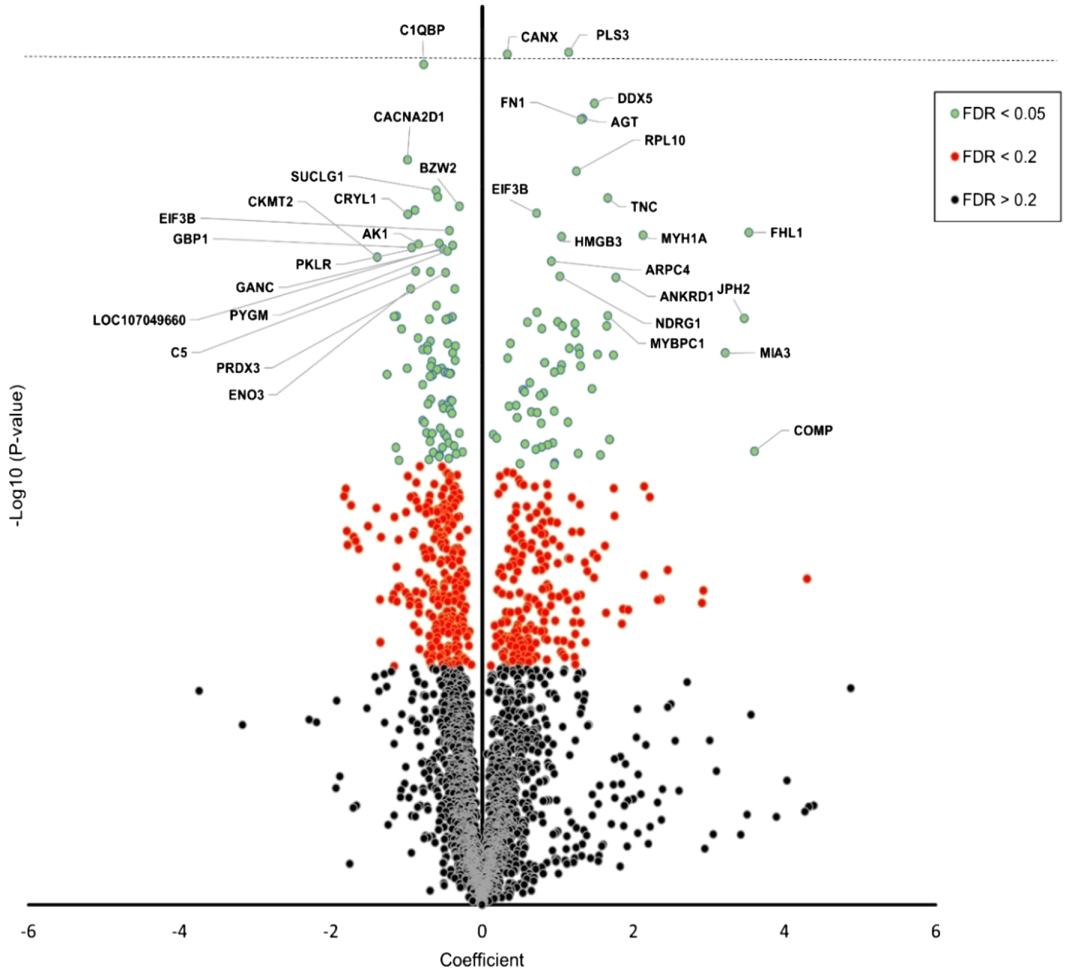

Figure 1. Volcano plot showing the differential expression of compounds in the spaghetti breast muscle of 8 wk old broiler chickens. The samples were collected at ~3 h post-slaughter and analyzed using proteomics technology. The level of significance was determined at an FDR of 0.05 (green dots) in the volcano plot and was separated by the horizontal dotted line.

Table 2. Expression of differentially expressed proteins in spaghetti meat.

Abbreviation	Full name	Expression
CANX	Calnexin	Upregulated
PLS3	Plastin 3	Upregulated
RPL10	Ribosomal protein L10	Upregulated
NDRG1	N-myc downstream regulated gene 1	Upregulated
CKMT2	Creatine kinase mitochondrial 2	Downregulated
C1QBP	Complement 1q binding protein C	Downregulated
EIF3B	Eukaryotic translation initiation factor 3 subunit B	Downregulated
ENO3	Enolase 3	Downregulated
PKLR	Pyruvate kinase L/R	Downregulated
PRDX3	Thioredoxin-dependent peroxide reductase mitochondrial	Downregulated
SUCLG1	Succinate-CoA ligase GDP/ADP-forming subunit alpha1	Downregulated
BZW2	Basic leucine zipper and w2 domains 2	Downregulated
CACNA2D1	Calcium voltage-gated channel auxiliary subunit alpha2delta 1	Downregulated

myopathies. Proteomic results for woody breast demonstrated dysfunctions in carbohydrate metabolism, protein synthesis, and calcium buffering capabilities in muscle cells (Kong et al., 2024). The differences in muscle proteomics showed higher oxidative stress in woody breast meat as well as negatively impacted ATP generation in muscles (Cai et al., 2018); this is similar to our previous studies, which also found that the ATP pathway is interrupted for woody breast fillets (Shakeri et al., 2023, 2024).

Many of the proteins found to be differentially expressed in SM samples in this study relate to cell and tissue health. Elevated PLS3 (T-plastin or fimbrin) (upregulated in SM) may play a role in cellular processes and signaling pathways. PLS3 has been reported to serve as a biomarker to determine cancer (induction of cell death), and mutations of PLS3 can cause osteoporosis (Wolff et al., 2021). Therefore, normal PLS3 expression seems to be essential for healthy cells. CANX (upregulated in SM) is a key factor in the quality control of proteins. It has been reported that CANX is upregulated during cellular stress and induces cell death (Guérin et al., 2008). Upregulated NDRG1 could be another sign of cellular stress (Chua et al., 2007). Additionally, RPL10 (upregulated in SM) regulates ROS in mitochondria, and an elevated level of RPL10 can be used as an indicator for cancer progress (Yang et al., 2018).

NDRG1 (downregulated in SM) is involved in the inhibition of multiple oncogenic signaling pathways. It plays a critical role in tumor metastasis suppression, and upregulation of the gene is linked to tumor growth (Chua et al., 2007). Furthermore, previous studies showed that downregulating both BZW2 and EIF3B (both downregulated in SM) could impair cell growth and promote tumor cell growth (Gao et al., 2019; Ma et al., 2019). The alteration in regulation of these proteins (NDRG1, BZW2, and EIF3B) might be related to mitochondria dysfunction as abnormal mitochondria could potentially promote tumors by altering ROS and Ca^{2+} (Hsu et al., 2016). Histologic investigations reported that the SM myopathy increases inflammatory cell infiltration and necrosis (Baldi et al., 2018), potentially contributing to tumor invasion, growth, and metastasis (Talmadge et al., 2007). In fact, when damaged cells grow and multiply, they may form tumors (Fink and Cookson, 2005).

CACNA2D1 (downregulated in SM) controls calcium channels (Cav1. 2 channels) which provides a mechanism to modulate the force of contraction (Cooper and Dimri, 2023). Mitochondria contribute to buffering cellular calcium levels by transporting calcium into mitochondria through channels. However, when the channels do not work properly, it promotes the opening of the mitochondrial permeability transition pore and triggers apoptotic cell death (Matuz-Mares et al., 2022). When cells are not stressed, mitochondrial permeability transition pore is closed and the water and solute passing protein channel impermeable to ions. Under oxidative stress conditions, sudden mitochondrial permeability transition pore opening causes massive ion influx that shuts down ATP production (Vaseva et al., 2012).

ENO3 and PKLR (both downregulated in SM) are related to glycogen metabolism, muscle development, and mitochondrial function (Liu et al., 2019; Cui et al., 2021; Nieborak et al., 2023). PKLR mainly is active in the liver and in red blood cells, which contain hemoglobin that carries oxygen to all parts of the body. PKLR is involved in producing an enzyme called pyruvate kinase, which is a key enzyme in glycolysis and energy production. Deficiency of PKLR could impair cell function and survival (van Wijk et al., 2003). Downregulating PKLR leads to decreased glucose uptake and mitochondrial activity (Liu et al., 2019), which is responsible for generating ATP for the body. ENO3 is a glycolytic enzyme that catalyzes the reversible conversion of 2-phosphoglycerate to phosphoenolpyruvate (second source of ATP in glycolysis). Downregulated ENO3 and PKLR in SM indicates a disruption in cellular energy production within the affected muscle tissue.

The impacts of SM on glucose metabolism could be related to mitochondria function as glucose converts to pyruvate, which enters mitochondria to undergo a further metabolic pathway, the tricarboxylic acid cycle

(Kwak et al., 2010). Several proteins related to mitochondria function including PRDX3, CKMT2, SUCLG1 and C1QBP were downregulated in SM samples, indicating that there is a potential disruption in energy production in SM tissue. Mitochondria generate ATP (main source of energy for cells) by utilizing the energy released from consumed foods (Brand et al., 2013). PRDX3 plays an important role in regulating oxidation-induced apoptosis as well as improving muscle strength and ATP production (Lee et al., 2014); thus, it can control the contractile functions of skeletal muscle. Furthermore, it helps maintain normal mitochondrial function and mitochondria DNA copy number as well as decreases oxidative damages (Ahn et al., 2022), suggesting that downregulating of PRDX3 induces oxidative stress and mitochondria impairment, leading to lower energy supply and cellular health (Zhang et al., 2016; Sonn et al., 2022). It is worth mentioning that PRDX3 is a mitochondrial antioxidant protein and eliminates high amounts of H_2O_2 in the mitochondria (Lee et al., 2014). CKMT2 encodes S-type creatine kinase mitochondria, responsible for transferring energy from mitochondria to creatine (Schlattner et al., 2006). SUCLG1 links to mitochondria DNA abnormalities causing several disorders in the body such as weak muscle (El-Hattab and Scaglia, 2017). C1QBP is located in the mitochondria and regulates cellular respiration (Wang et al., 2022a). C1QBP deficiency is involved in many cellular processes, including mitochondrial homeostasis, mitochondria oxidative phosphorylation, inflammation, and cancer (Wang et al., 2022a).

Conclusion

Based on the differentially expressed proteins identified in this study, we can conclude that abnormal mitochondrial activity may potentially play a role in the development of the SM myopathy by disrupting ATP production and increasing cell death as evidenced by upregulating stress compounds and downregulating compounds related to mitochondria function. Although this study provided valuable information regarding possible mechanisms involved in SM development (glucose pathway and mitochondria function), further investigations are required to fully understand the mechanisms and their connections to other vital pathways.

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Literature Cited

Ahn, B., Ranjit, R., Kneis, P., Xu, H., Piekarz, K. M., Freeman, W. M., Kinter, M., Richardson, A., Ran, Q., and Brooks, S. V. 2022. Scavenging mitochondrial hydrogen peroxide by peroxiredoxin 3 overexpression attenuates contractile dysfunction and muscle atrophy in a murine model of accelerated sarcopenia. Aging Cell 21:e13569. https://doi.org/10.1111/acel.13569

Baldi, G., Soglia, F., Mazzoni, M., Sirri, F., Canonico, L., Babini, E., Laghi, L., Cavani, C., and Petracci, M. 2018. Implications of white striping and spaghetti meat abnormalities on meat quality and histological features in broilers. Animal 12:164–173. https://doi.org/10.1017/S1751731117001069

Baldi, G., Soglia, F., and Petracci, M. 2021. Spaghetti meat abnormality in broilers: Current understanding and future research directions. Front. Physiol. 12:684497. https://doi.org/10.3389/fphys.2021.684497

Brand, M., Orr, A., Perevoshchikova, I., and Quinlan, C. 2013. The role of mitochondrial function and cellular bioenergetics in ageing and disease. Brit. J. Dermatol. 169:1–8. https://doi.org/10.1111/bjd.12208

Cai, K., Shao, W., Chen, X., Campbell, Y., Nair, M., Suman, S., Beach, C., Guyton, M., and Schilling, M. 2018. Meat quality traits and proteome profile of woody broiler breast (pectoralis major) meat. Poultry Sci. 97:337–346. https://doi.org/10.3382/ps/pex284

Chen, J., Wang, J., Wu, X., Simon, N., Svensson, C. I., Yuan, J., Hart, D. A., Ahmed, A. S., and Ackermann, P. W. 2023. Eef2 improves dense connective tissue repair and healing outcome by regulating cellular death, autophagy, apoptosis, proliferation and migration. Cell. Mol. Life Sci. 80:128. https://doi.org/10.1007/s00018-023-04776-x

Chua, M.-S., Sun, H., Cheung, S. T., Mason, V., Higgins, J., Ross, D. T., Fan, S. T., and So, S. 2007. Overexpression of NDRG1 is an indicator of poor prognosis in hepatocellular carcinoma. Modern Pathol. 20:76–83. https://doi.org/10.1038/modpathol.3800711

Clark, D., and Velleman, S. 2016. Spatial influence on breast muscle morphological structure, myofiber size, and gene expression associated with the wooden breast myopathy in broilers. Poultry Sci. 95:2930–2945. https://doi.org/10.3382/ps/pew243

Cooper, D., and Dimri, M. 2023. Biochemistry, calcium channels. In: StatPearls. StatPearls Publishing, Treasure Island, FL.

Cui, H., Guo, D., Zhang, X., Zhu, Y., Wang, Z., Jin, Y., Guo, W., and Zhang, S. 2021. ENO3 inhibits growth and metastasis of hepatocellular carcinoma via Wnt/β-catenin signaling pathway. Frontiers in Cell and Developmental Biology 9:797102. https://doi.org/10.3389/fcell.2021.797102

El-Hattab, A. W., and Scaglia, F. 2017. SUCLG1-related mitochondrial DNA depletion syndrome, encephalomyopathic form with methylmalonic aciduria. In: M. P. Adam, J. Feldman, G. M. Mirzaa, R. A. Pagon, S. E. Wallace, and A. Amemiya (editors), GeneReviews. University of Washington, Seattle, WA. https://www.ncbi.nlm.nih.gov/books/NBK425223/

Fink, S. L., and Cookson, B. T. 2005. Apoptosis, pyroptosis, and necrosis: Mechanistic description of dead and dying eukaryotic cells. Infect. Immun. 73:1907–1916. https://doi.org/10.1128/iai.73.4.1907-1916.2005

Gao, H., Yu, G., Zhang, X., Yu, S., Sun, Y., and Li, Y. 2019. BZW2 gene knockdown induces cell growth inhibition, G1 arrest and apoptosis in muscle-invasive bladder cancers: A microarray pathway analysis. J. Cell. Mol. Med. 23:3905–3915. https://doi.org/10.1111/jcmm.14266

Guérin, R., Arseneault, G., Dumont, S., and Rokeach, L. A. 2008. Calnexin is involved in apoptosis induced by endoplasmic reticulum stress in the fission yeast. Mol. Biol. Cell. 19:4404–4420. https://doi.org/10.1091/mbc.e08-02-0188

Hsu, C.-C., Tseng, L.-M., and Lee, H.-C. 2016. Role of mitochondrial dysfunction in cancer progression. Exp. Biol. Med. 241:1281–1295. https://doi.org/10.1177/1535370216641787

Hug, N., Longman, D., and Cáceres, J. F. 2016. Mechanism and regulation of the nonsense-mediated decay pathway. Nucleic Acids Res. 44:1483–1495. http://doi10.1093/nar/gkw010

Kong, B., Owens, C., Bottje, W., Shakeri, M., Choi, J., Zhuang, H., and Bowker, B. 2024. Proteomic analyses on chicken breast meat with white striping myopathy. Poultry Sci. 103:103682. https://doi.org/10.1016/j.psj.2024.103682

Kuttappan, V., Hargis, B., and Owens, C. 2016. White striping and woody breast myopathies in the modern poultry industry: A review. Poultry Sci. 95:2724–2733. https://doi.org/10.3382/ps/pew216

Kwak, S. H., Park, K. S., Lee, K. U., and Lee, H. K. 2010. Mitochondrial metabolism and diabetes. J. Diabetes Invest. 1:161–169. https://doi.org/10.1111/j.2040-1124.2010.00047.x

Lee, K.-P., Shin, Y. J., Cho, S. C., Lee, S.-M., Bahn, Y. J., Kim, J. Y., Kwon, E.-S., Park, S. C., Rhee, S. G., and Woo, H. A. 2014. Peroxiredoxin 3 has a crucial role in the contractile function of skeletal muscle by regulating mitochondrial homeostasis. Free Radical Bio. Med. 77:298–306. https://doi.org/10.1016/j.freeradbiomed.2014.09.010

Liu, Z., Zhang, C., Lee, S., Kim, W., Klevstig, M., Harzandi, A. M., Sikanic, N., Arif, M., Ståhlman, M., and Nielsen, J. 2019. Pyruvate kinase L/R is a regulator of lipid metabolism and mitochondrial function. Metab. Eng. 52:263–272. https://doi.org/10.1016/j.ymben.2019.01.001

Ma, F., Li, X., Ren, J., Guo, R., Li, Y., Liu, J., Sun, Y., Liu, Z., Jia, J., and Li, W. 2019. Downregulation of eukaryotic translation initiation factor 3b inhibited proliferation and metastasis of gastric cancer. Cell Death Dis. 10:623. https://doi.org/10.1038/s41419-019-1846-0

Matuz-Mares, D., González-Andrade, M., Araiza-Villanueva, M. G., Vilchis-Landeros, M. M., Vázquez-Meza, H. 2022. Mitochondrial calcium: Effects of its imbalance in disease. Antioxidants 11:801. https://doi.org/10.3390/antiox11050801

Nickless, A., Bailis, J. M., and You, Z. 2017. Control of gene expression through the nonsense-mediated RNA decay pathway. Cell Biosci. 7:1–12. https://doi.org/10.1186/s13578-017-0153-7

Nieborak, A., Lukauskas, S., Capellades, J., Heyn, P., Santos, G. S., Motzler, K., Zeigerer, A., Bester, R., Protzer, U., and Schelter, F. 2023. Depletion of pyruvate kinase (PK) activity causes glycolytic intermediate imbalances and reveals a PK-TXNIP regulatory axis. Mol. Metab. 101748. https://doi.org/10.1016/j.molmet.2023.101748

Petracci, M., Soglia, F., Madruga, M., Carvalho, L., Ida, E., and Estévez, M. 2019. Wooden-breast, white striping, and spaghetti meat: Causes, consequences and consumer perception of emerging broiler meat abnormalities. Compr. Rev. Food Sci. F. 18:565–583. https://doi.org/10.1111/1541-4337.12431

Schlattner, U., Tokarska-Schlattner, M., and Wallimann, T. 2006. Mitochondrial creatine kinase in human health and disease. BBA-Mol. Basis Dis. 1762:164–180. https://doi.org/10.1016/j.bbadis.2005.09.004

Shakeri, M., Choi, J., Harris, C., Buhr, R. J., Kong, B., Zhuang, H., and Bowker, B. 2024. Reduced ribonucleotide reductase RRM2 subunit expression increases DNA damage and mitochondria dysfunction in woody breast chickens. Am. J. Vet. Res. 1:1–7. https://doi.org/10.2460/ajvr.23.12.0283

Shakeri, M., Kong, B., Zhuang, H., and Bowker, B. 2023. Potential role of ribonucleotide reductase enzyme in mitochondria function and woody breast condition in broiler chickens. Animals 13:2038. https://doi.org/10.3390/ani13122038

Soglia, F., Laghi, L., Canonico, L., Cavani, C., and Petracci, M. 2016. Functional property issues in broiler breast meat related to emerging muscle abnormalities. Food Res. Int. 89:1071–1076. https://doi.org/10.1016/j.foodres.2016.04.042

Sonn, S. K., Song, E. J., Seo, S., Kim, Y. Y., Um, J.-H., Yeo, F. J., Jeon, S., Lee, M.-N., Jin, J., and Kweon, H. Y. 2022. Peroxiredoxin 3 deficiency induces cardiac hypertrophy and dysfunction by impaired mitochondrial quality control. Redox Biol. 51:102275. https://doi.org/10.1016/j.redox.2022.102275

Talmadge, J. E., Donkor, M., and Scholar, E. 2007. Inflammatory cell infiltration of tumors: Jekyll or Hyde. Cancer Metast. Rev. 26:373–400. https://doi.org/10.1007/s10555-007-9072-0

van Wijk, R., van Solinge, W. W., Nerlov, C., Beutler, E., Gelbart, T., Rijksen, G., and Nielsen, F. C. 2003. Disruption of a novel regulatory element in the erythroid-specific promoter of the human PKLR gene causes severe pyruvate kinase deficiency. Blood 101:1596–1602. https://doi.org/10.1182/blood-2002-07-2321

Vaseva, A. V., Marchenko, N. D., Ji, K., Tsirka, S. E., Holzmann, S., and Moll, U. M. 2012. p53 opens the mitochondrial permeability transition pore to trigger necrosis. Cell 149:1536–1548. https://doi.org/10.1016/j.cell.2012.05.014

Wagle, B. R., Quach, A., Yeo, S., Assumpcao, A. L., Arsi, K., Donoghue, A. M., and Jesudhasan, P. R. 2023. A multiomic

analysis of chicken serum revealed the modulation of host factors due to *Campylobacter jejuni* colonization and in-water supplementation of eugenol nanoemulsion. Animals 13:559. https://doi.org/10.3390/ani13040559

Wang, J., Huang, C. L., and Zhang, Y. 2022a. Complement C1q binding protein (C1QBP): Physiological functions, mutation-associated mitochondrial cardiomyopathy and current disease models. Frontiers in Cardiovascular Medicine 9:843853. https://doi.org/10.3389/fcvm.2022.843853

Wang, L., Yang, Z., He, X., Pu, S., Yang, C., Wu, Q., Zhou, Z., Cen, X., and Zhao, H. 2022b. Mitochondrial protein dysfunction in pathogenesis of neurological diseases. Front. Mol. Neurosci. 15:974480. https://doi.org/10.3389/fnmol.2022.974480

Waterhouse, N. J. 2003. The cellular energy crisis: Mitochondria and cell death. Med. Sci. Sport. Exer. 35:105–110. https://doi.org/10.1097/00005768-200301000-00017

Wolff, L., Strathmann, E. A., Müller, I., Mählich, D., Veltman, C., Niehoff, A., and Wirth, B. 2021. Plastin 3 in health and disease: A matter of balance. Cell. Mol. Life Sci. 78:5275–5301. https://doi.org/10.1007/s00018-021-03843-5

Yang, J., Chen, Z., Liu, N., and Chen, Y. 2018. Ribosomal protein l10 in mitochondria serves as a regulator for ros level in pancreatic cancer cells. Redox Biol. 19:158–165. https://doi.org/10.1016/j.redox.2018.08.016

Zhang, Y.-G., Wang, L., Kaifu, T., Li, J., Li, X., and Li, L. 2016. Featured article: Accelerated decline of physical strength in peroxiredoxin-3 knockout mice. Exp. Biol. Med. 241:1395–1400. https://doi.org/10.1177/1535370216642039