UNIVERSIDADE DO ESTADO DE SANTA CATARINA – UDESC
CENTRO DE EDUCAÇÃO SUPERIOR DO OESTE – CEO
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA – PPGZOO

MARIANE SPUDEIT DAL PIZZOL

A INFLUÊNCIA DE microRNAs NA MANIFESTAÇÃO DAS MIOPATIAS PEITORAIS *WHITE STRIPING* E *WOODEN BREAST* EM FRANGOS DE CORTE

CHAPECÓ
2023

MARIANE SPUDEIT DAL PIZZOL

A INFLUÊNCIA DE microRNAs NA MANIFESTAÇÃO DAS MIOPATIAS PEITORAIS *WHITE STRIPING* E *WOODEN BREAST* EM FRANGOS DE CORTE

Dissertação apresentada como requisito parcial para obtenção do título de mestre em Zootecnia pelo Programa de Pós-Graduação em Zootecnia da Universidade do Estado de Santa Catarina – UDESC.
Orientador (a): Prof. Dra. Mônica Corrêa Ledur.
Coorientador (a): Dra. Adriana M. G. Ibelli

CHAPECÓ

2023

Ficha catalográfica elaborada pelo programa de geração automática da Biblioteca Setorial do CEO/UDESC, com os dados fornecidos pelo(a) autor(a)

Spudeit Dal Pizzol, Mariane
A INFLUÊNCIA DE microRNAs NA MANIFESTAÇÃO DAS MIOPATIAS PEITORAIS WHITE STRIPING E WOODEN BREAST EM FRANGOS DE CORTE / Mariane Spudeit Dal Pizzol. -- 2023.
102 p.

Orientadora: Mônica Corrêa Ledur
Coorientadora: Adriana Mércia Guaratini Ibelli
Dissertação (mestrado) -- Universidade do Estado de Santa Catarina, Centro de Educação Superior do Oeste, Programa de Pós-Graduação em Zootecnia, Chapecó, 2023.

1. Epigenética. 2. Avicultura. 3. Miopatias Peitorais. 4. microRNAs. 5. Melhoramento Genético. I. Corrêa Ledur, Mônica . II. Guaratini Ibelli, Adriana Mércia. III. Universidade do Estado de Santa Catarina, Centro de Educação Superior do Oeste, Programa de Pós-Graduação em Zootecnia. IV. Titulo.

MARIANE SPUDEIT DAL PIZZOL

A INFLUÊNCIA DE microRNAs NA MANIFESTAÇÃO DAS MIOPATIAS PEITORAIS *WHITE STRIPING* E *WOODEN BREAST* EM FRANGOS DE CORTE

Dissertação apresentada como requisito parcial para obtenção do título de mestre em Zootecnia pelo Programa de Pós-Graduação em Zootecnia, da Universidade do Estado de Santa Catarina – UDESC.
Orientador: Prof. Dra. Mônica Corrêa Ledur
Coorientador: Dra. Adriana M. G. Ibelli

BANCA EXAMINADORA

Membros:

Dra. Mônica Côrrea Ledur
(Embrapa Suínos e Aves/UDESC-Oeste)

Dr. Rogério Ferreira
(UDESC-Oeste)

Dr. Maurício Egídio Cantão
(Embrapa Suínos e Aves)

Chapecó, 07 de fevereiro de 2023.

AGRADECIMENTOS

Primeiramente, sou grata por todo apoio da minha família, em especial minha mãe Augusta Rosana Spudeit, pelo incentivo, suporte e amor durante toda a minha vida e durante o mestrado. Amo você de todo o coração, obrigada por ser essa mãe maravilhosa.

Agradeço a minha orientadora Mônica Côrrea Ledur, e minha coorientadora Adriana Ibelli, por toda a ajuda, paciência, orientação e amizade, e acima de tudo por terem acreditado em mim. Pessoas pelas quais eu sinto profunda admiração e carinho, me orgulho de ter participado dessa equipe.

Sou muito grata a todas as pessoas próximas e amigos que torceram pelo meu sucesso, que estiveram do meu lado nos momentos de felicidade, tristeza e desespero que passei com o mestrado, e ao meu companheiro Douglas Scariot, que me ajudou e me apoiou desde o início, corrigindo meus textos, me auxiliando com a bioinformática, dando suporte emocional e de infraestrutura eletrônica.

Agradeço também às instituições que me receberam de braços abertos, Embrapa Suínos e Aves e UDESC-Oeste, e principalmente aos colegas do laboratório de genética, que foram como uma família pra mim e tornaram meu processo mais leve e alegre.

Agradeço à FAPESC, agência de fomento que me concedeu bolsa possibilitando que eu cursasse o programa de pós-graduação.

“O método científico é comprovado e verdadeiro. Não é perfeito, é apenas o melhor que temos. Abandoná-lo, junto com seus protocolos céticos, é o caminho para uma idade das trevas.” (Carl Sagan)

RESUMO

As miopatias peitorais em frangos de corte têm aumentado substancialmente nas últimas décadas, se tornando um problema complexo para o setor. As miopatias que mais se destacam são *White Striping* (WS) e *Wooden Breast* (WB). Estas desordens são de natureza degenerativa e podem afetar até 96% dos animais. A genética foi correlacionada com o desenvolvimento dessas miopatias, entretanto, ainda não é esclarecido o papel dos fatores epigenéticos na manifestação e diferenciação dessas condições. Assim, o objetivo deste estudo foi identificar o perfil de expressão diferencial de microRNAs (miRNAs) entre *White Striping*, *Wooden Breast* e o grupo controle, assim como, verificar o potencial de atuação destes miRNAs sobre vias metabólicas relacionadas com a ocorrência das miopatias. Utilizamos 14 frangos com 28 dias de idade (3 controles, 5 afetados com WS e 6 afetados com WB) para as análises de sequenciamento. A extração de RNA foi feita com Trizol, as bibliotecas foram feitas com o kit TruSeq Stranded SmallRNA e o sequenciamento foi realizado em equipamento NextSeq (*single-end*). As leituras passaram por controle de qualidade e seguiram para identificação, contagem e mapeamento de miRNAs. Os miRNAs obtidos foram analisados para expressão diferencial e seus alvos foram preditos; os genes resultantes seguiram para análise funcional. Ao todo, foram detectados 303 miRNAs entre todas as amostras, sendo 286 conhecidos e 17 novos. A expressão diferencial resultou em 5 miRNAs DE para WS vs controle, 82 miRNAs DE para WB vs controle e 61 miRNAs DE entre WB e WS. A análise funcional revelou 6 vias significativas em comum para os genes controlados por miRNAs DE das duas miopatias, sendo elas: autofagia, via de sinalização da insulina, via de sinalização *FoxO*, ciclo celular, endocitose e vias metabólicas. Duas vias foram significativas exclusivamente para a comparação de WS vs controle, que são via de sinalização *ERBB* e via de sinalização *mTOR*, e para WB vs controle foram identificadas 14 vias exclusivas, das quais a discussão se aprofundou apenas nas duas mais significativas, que foram a proteólise mediada por ubiquitina e o processamento de proteínas no retículo endoplasmático. Nosso estudo confirmou a presença de miRNAs já relacionados com o desenvolvimento de WB, entre eles o miR-155, miR-146b, miR-133 e miR-222. Identificamos também miRNAs que estão sendo relacionados com WS e WB pela primeira vez, como miR-15b, miR-130, miR-30, miR-200, miR-429, miR-375 entre outros. Evidenciamos a capacidade de

atuação destes miRNAs em vias metabólicas relevantes para o desenvolvimento de WS e WB, regulando genes envolvidos com hipóxia, apoptose, inflamação e proliferação celular. Desta maneira, foi possível concluir que existem miRNAs com potencial de influenciar características no músculo peitoral de frangos de corte que estão relacionados com o desenvolvimento de WS e WB.

Palavras-chave: Epigenética; Avicultura; Miopatias Peitorais; miRNAs; Melhoramento Genético.

ABSTRACT

The occurrence of chicken pectoral myopathies has increased substantially in recent years, becoming a complex problem for the poultry production. The most common myopathies are White Striping (WS) and Wooden Breast (WB), which are degenerative disorders and can affect up to 96% of animals. It is known that there is a genetic component involved with the development of these myopathies. However, the role of epigenetic regulation in the manifestation and differentiation of these conditions is still unclear. Thus, the aim of this study was to identify microRNAs (miRNAs), and also verify if they are differentially expressed (DE) between the two myopathies and the control group. These findings could highlight miRNAs, their target genes and biological pathways involved with these conditions. A total of 14 chickens with 28 days of age (3 controls, 5 affected with WS and 6 affected with WB) were used for the sequencing analysis. RNA extraction was performed with Trizol and then, libraries were prepared with the TruSeq Stranded SmallRNA kit (Illumina). The sequencing was performed on NextSeq equipment with a single-end protocol. Reads were submitted to quality control, mapping, miRNA identification and counting. The obtained miRNAs were analyzed for differential expression and their targets were predicted and used for functional analysis. A total of 303 miRNAs were detected among all samples, 286 known and 17 new. Differential expression resulted in 5 DE miRNAs for WS vs control, 82 DE miRNAs for WB vs control, and 61 DE miRNAs between WB and WS. Functional analysis revealed 6 significant pathways in common for genes controlled by DE miRNAs from both myopathies, namely: autophagy, insulin signaling pathway, FoxO signaling pathway, cell cycle, endocytosis and metabolic pathways. Two pathways were exclusively significant for the comparison of WS vs control, which are ERBB signaling and *mTOR* signaling pathway. For WB vs control, 14 unique pathways were identified, of which only the two most significant were discussed: the ubiquitin-mediated proteolysis and protein processing in the endoplasmic reticulum. Our study confirmed the presence of miRNAs already related to the development of WB, including miR-155, miR-146b, miR-133 and miR-222. We also identified miRNAs that are being related to WS and WB for the first time, such as miR-15b, miR-130, miR-30, miR-200, miR-429, miR-375, among others. We showed that the role of these miRNAs is important for WS and WB development. It was observed that miRNA target genes are regulating

hypoxia, apoptosis, inflammation and cell proliferation. Some of these molecular mechanisms have already been described acting on pectoral myopathies. Therefore, it was possible to conclude that miRNAs are acting in the pectoral muscle of broilers and are involved in the WS and WB occurence.

Keywords: Epigenetics; Poultry farming; Pectoral Myopathies; miRNAs; animal breeding.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

C	Controle
DE	Diferencialmente Expressos
dsRNA	RNA Dupla-fita
FDR	False Discovery Rate
GO	Ontologia Gênica
MDS	Multidimensional Scaling Plot
miRNA	microRNA
MP	Membrana Plasmática
mRNA	RNA Mensageiro
Nt	Nucleotídeos
PMM	Músculo *Pectoralis Major*
QC	Controle de Qualidade
RE	Retículo Endoplasmático
RIN	Número de Integridade de RNA
RTK	Receptores de Tirosina Quinase
siRNA	Pequeno RNA de Interferência
SNP	Polimorfismo de Base Única
UMI	Identificadores Moleculares Únicos
UP	Ubiquitina-proteassoma
UPR	Resposta de Proteína Mal Dobrada
WB	*Wooden Breast*
WS	*White Striping*

SUMÁRIO

1 INTRODUÇÃO

A produção animal vem se aperfeiçoando ao longo do tempo para atender o crescimento da demanda alimentícia mundial. A proteína animal tem grande importância na alimentação humana, e os maiores aliados na produção animal têm sido as ferramentas de melhoramento genético, em conjunto com o desenvolvimento de áreas como a nutrição, sanidade e manejo.

O melhoramento atua há décadas na seleção de características economicamente importantes para a indústria, como por exemplo, ganho de peso, conversão alimentar e qualidade da carne. Na avicultura de corte, percebe-se que o foco do rendimento de carcaça estava voltado especialmente para o rendimento do músculo peitoral (PETRACCI *et al*., 2015), isso devido ao valor comercial desse corte.

Os programas de melhoramento genético tradicionais de frangos de corte tiveram um grande avanço nas últimas décadas, de modo que se estimou que de 1957 até 2005, o crescimento dos animais aumentou em 400%, alcançando uma redução de 50% na taxa de conversão alimentar (ZUIDHOF *et al*., 2014). Atualmente contamos com animais altamente produtivos, mas esse fato veio acompanhado de um aumento substancial nos casos de distúrbios metabólicos; essas desordens já vêm sendo relatadas há algum tempo (SCHEELE, 1997), e no momento atual elas tem guiado os processos de seleção genética a buscarem características de manutenção fisiológica das aves.

Uma das grandes preocupações da agroindústria na atualidade é a alta incidência de miopatias peitorais em frangos de corte. Esses distúrbios têm tido um aumento substancial de casos na última década, alguns autores relatam 10% de animais acometidos por WS (FERREIRA *et al*., 2014), enquanto outros grupos já apontam a prevalência de 25,7% (RUSSO *et al*., 2015), 96,1% de animais afetados com o estágio inicial, 32,3% de animais com o estágio moderado e 2% com o estágio severo (TIJARE *et al*., 2016). Os filés acometidos pelas miopatias não causam risco à saúde do consumidor, pois não se tratam de uma condição infecciosa, porém provocam uma maior rejeição do público comprador relacionada diretamente ao aspecto desagradável da carne (KUTTAPPAN *et al*., 2012a), as miopatias são distúrbios degenerativos que afetam o músculo peitoral das aves (KUTTAPPAN *et al*., 2012b). Essa rejeição se soma a perda de qualidade da

composição química da carne (SIHVO; IMMONEN; PUOLANNE, 2014; DE ALMEIDA ASSUNÇÃO *et al.,* 2020; XING *et al*., 2021), alterações na exsudação e deposição de colágeno no tecido trazem complicações no processamento desses cortes pela indústria (BRAMBILA; BOWKER; ZHUANG, 2016; PRAUD *et al*., 2020; OLIVEIRA *et al*., 2021). Casos mais severos dos distúrbios também são responsáveis pela condenação de carcaças nos abatedouros (ZANETTI *et al*., 2018). Dessa maneira, as miopatias peitorais vêm trazendo prejuízos econômicos para toda a cadeia produtiva de frangos de corte (KUTTAPPAN *et al.,* 2012a; ZANETTI *et al*., 2018), se caracterizando como distúrbios complexos que precisam ser melhor entendidos para serem solucionados.

Entre as principais miopatias que afetam os frangos de corte, destacam-se duas, a *Wooden Breast* (WB) e a *White Striping* (WS). A etiologia desses distúrbios ainda precisa ser esclarecida, mas diversos estudos como o de Marciano *et al*. (2021) já identificaram alguns genes relacionados ao desenvolvimento de WS, e também existem evidências de genes ligados às alterações associadas com WB e WS (BORDINI *et al*., 2022). Além dos mecanismos genéticos envolvidos com o desencadeamento das miopatias, os mecanismos epigenéticos atuam de forma significativa na expressão proteica dos animais e também podem estar envolvidos com a manifestação desses distúrbios.

Entre os processos envolvidos no controle da expressão gênica, destacam-se os complexos de silenciamento gênico pós-transcricional guiados por microRNAs (miRNAs). Este mecanismo é capaz de degradar RNAs mensageiros impedindo que eles se expressem (GEBERT; MACRAE, 2019), e desregulações dessa via podem gerar modificações biológicas que resultam na manifestação de distúrbios como as miopatias.

Já existem evidências da atuação de miRNAs em frangos de corte com 42 dias de idade afetados com WB (SHU *et al*., 2021). Entretanto, esses mecanismos precisam ser mais bem explorados visando à identificação dos mecanismos epigenéticos atrelados ao aparecimento das miopatias, o que pode ser possível em uma fase mais inicial dos distúrbios. Assim, este estudo objetivou investigar a diferença no perfil de expressão de miRNAs entre animais normais e animais afetados com *White Striping* e *Wooden* Breast, e o potencial de atuação destes miRNAs em vias metabólicas que podem estar ligadas com o desencadeamento das miopatias peitorais em frangos de corte com 28 dias de idade.

2 REVISÃO BIBLIOGRÁFICA

2.1 AVICULTURA MODERNA

A produção avícola mudou drasticamente nos últimos 50 anos, com o aprimoramento da nutrição e manejo e a intensificação da seleção genética. Porém, atualmente são conhecidos alguns problemas que vêm atrelados a esse alto desempenho, como as desordens metabólicas. A cadeia avícola está buscando alternativas para solucionar esses problemas, sem diminuir a produtividade e ainda atender as normas de bem-estar animal e andar em direção a uma produção mais sustentável (GRIFFIN *et al*., 2017).

A carne de frango é uma fonte de proteínas importante na alimentação humana, por isso, ao longo do tempo foram somando-se tecnologias e conhecimentos na área de genética, nutrição, manejo e ambiência para maximizar a eficiência da produção avícola (ZUIDHOF *et al*., 2014). No Brasil, a avicultura representa um setor importante da economia. Em 2019 a produção foi maior que 13 milhões de toneladas de carne de frango, das quais 32% foram destinadas para exportação, com receita aproximada de 7 bilhões de dólares (ABPA, 2021).

Ao longo do tempo, a indústria foi modificando seus produtos de acordo com o perfil dos consumidores e houve a valorização de alguns cortes, como o peito de frango, que tem baixo valor calórico e é relativamente barato (PETRACCI *et al*., 2015). A partir disso, programas de melhoramento vêm atuando na seleção genética buscando atingir maiores pesos do músculo peitoral em frangos de corte, para atender a demanda comercial (PETRACCI *et al*., 2015).

Os avanços científicos e tecnológicos possibilitaram a aumento da produção e diminuição das perdas em todo o processo, elevando a produtividade a patamares nunca atingidos antes. Assim como a forma de produzir alimentos foi se modificando ao longo do tempo, o perfil dos consumidores também foi mudando de acordo com suas necessidades. Por isso, a cadeia produtiva, que é muito competitiva, está constantemente sendo modificada para se manter alinhada com os interesses do mercado.

A cadeia produtiva do agronegócio representa uma parcela significativa da economia nacional, sendo que em 2010 a produção nacional de carne de frango era de 12,230 milhões de toneladas, enquanto em 2020 a produção alcançou 13,845

milhões de toneladas (ABPA, 2021). A eficiência produtiva da avicultura atualmente permite ao Brasil suprir o mercado interno, e em 2020, 69% da produção brasileira de carne de frango atendeu esse setor, enquanto 31% foi destinada à exportação (ABPA, 2021).

Esse aumento na produção se faz necessário em decorrência do consumo per capita que vem aumentando, assim como o tamanho da população. O número de habitantes no Brasil em 2022 passou de 214 milhões (IBGE, 2022), e enquanto o consumo per capita de carne de frango em 2010 era de aproximadamente 44,09 kg/hab, em 2020 houve um aumento para 45,27 kg/hab (ABPA, 2021).

A produtividade aumentou exponencialmente nas últimas décadas sendo que o crescimento de frangos de corte aumentou em 400% de 1957 a 2005, assim como houve a redução de 50% na taxa de conversão alimentar (ZUIDHOF *et al*., 2014). Atualmente, com esse aumento no crescimento, o músculo peitoral menor desses animais também teve um rendimento 30% maior em machos e 37% maior nas fêmeas aos 42 dias, enquanto o músculo peitoral maior aumentou 79% nos machos e 85% nas fêmeas (ZUIDHOF *et al*., 2014).

Essa alta eficiência metabólica dos frangos de corte é obtida graças aos rigorosos programas de melhoramento genético, em combinação com uma nutrição de ponta e manejo especializado, essas áreas juntas, permitem um maior desempenho dos animais (ZUIDHOF *et al*., 2014). Aliado com esse grande desempenho alcançado através das melhorias dos sistemas de produção, foram surgindo alguns problemas que acarretam prejuízos econômicos e ao bem-estar animal. Os animais de produção intensiva passam por algumas restrições comportamentais, que desafiam as aves e tipicamente resultam em estresse. O estresse por sua vez pode contribuir para o aparecimento de diversos distúrbios metabólicos, como a ascite, claudicação, problemas esqueléticos, dermatites e miopatias (DE JONG *et al*., 2012).

As complicações não previstas geradas pelo processo de seleção se tornaram um grande desafio para a avicultura de corte, sendo necessário modificar as características para seleção nos programas de melhoramento genético. O objetivo do momento é alcançar animais equilibrados fisiologicamente (ZUIDHOF *et al*., 2014).

Várias desordens metabólicas vêm sendo correlacionadas com a taxa de rendimento do músculo peitoral, e as miopatias que mais se destacam são a WB e

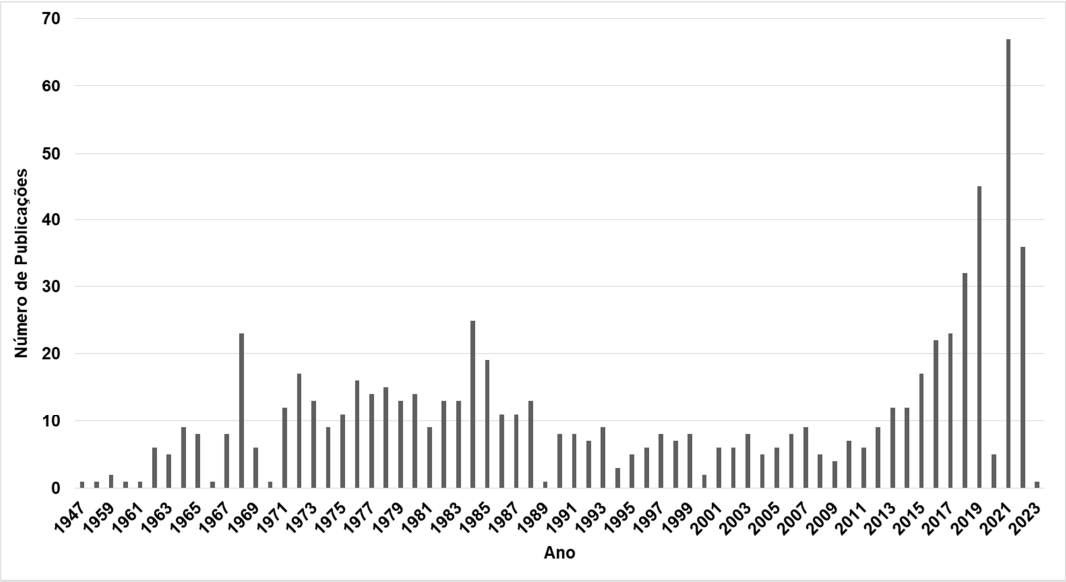
WS (KUTTAPPAN *et al*., 2012b). A WB é uma miopatia caracterizada pelo enrijecimento e miodegeneração do músculo peitoral das aves, que reduz a qualidade nutricional da carne e afeta a aceitação pelo consumidor (SIHVO; IMMONEN; PUOLANNE; 2014). *White Striping* é uma miopatia crônica degenerativa dos músculos peitorais que está se tornando cada vez mais preocupante, pois afeta negativamente o aspecto e a composição química do peito de frango, trazendo diversas perdas para a cadeia de produção (KUTTAPPAN *et al*., 2013a).

Após o primeiro projeto de montagem do genoma da galinha (International Chicken Genome Sequencing Consortium, 2004), as estratégias do melhoramento foram se tornando mais complexas e o conhecimento gerado vem sendo usado para diversas aplicações (BURT, 2004). Isso tornou possível um avanço substancial na avicultura, levando a ciência e tecnologia aplicada a toda cadeia de produção. Várias áreas surgiram a partir da biologia molecular, como a genômica, transcriptômica, proteômica e metabolômica. Pesquisas na área buscam identificar genes e investigar suas funções, assim como descobrir as diferenças a nível molecular que possam desencadear certas doenças entre várias outras aplicações.

2.2 MIOPATIAS PEITORAIS

As miopatias peitorais são alterações degenerativas que ocorrem no músculo *pectoralis major* (PMM) das aves, afetando a função das fibras musculares (KUTTAPPAN *et al*., 2012b) e também outras características. As miopatias peitorais têm sido um dos grandes desafios da avicultura de corte mundial. Esta relevância pode ser percebida pelo número de publicações relacionadas a este tema nos últimos 5 anos. Em uma busca por “chicken myopathy” no site do pubmed (https://pubmed.ncbi.nlm.nih.gov/) em janeiro de 2023, foi possível verificar que 40% das publicações foram disponibilizadas neste período, em diversas áreas como qualidade da carne, nutrição, diagnóstico, biologia molecular, entre outras (**Figura 1**).

Figura 1 - *Número de publicações sobre miopatias em galinhas por ano desde 1947 até janeiro de 2023*



Fonte: Pubmed.

As miopatias peitorais, incluindo *White Striping* (WS) e *Wooden Breast* (WB), têm se demonstrado cada vez mais frequentes na avicultura moderna. O peito de frango é um corte muito importante economicamente, mas sob pressão de forte seleção genética que busca um crescimento muito acelerado e com alto rendimento do músculo peitoral, acabou levando ao desenvolvimento de alguns problemas fisiológicos que ocasionam estrias brancas no músculo peitoral das aves (WS) e o peito amadeirado (WB). Apesar de não representar um risco alimentar, a carne de animais afetados pelas miopatias peitorais tem qualidade reduzida (MUDALAL *et al*., 2014), e o aspecto da carne também tende a ser desagradável afetando a aceitabilidade dos filés pelo mercado consumidor (DE ALMEIDA ASSUNÇÃO *et al*., 2020).

Assim, essas miopatias afetam o aspecto da carne, o valor nutricional, o desempenho da carne ao processamento e a aceitabilidade dos consumidores, acarretando diversos prejuízos para a cadeia de produção (PRAUD *et al*., 2020).

2.3 *WHITE STRIPING*

A miopatia peitoral WS se caracteriza por estrias esbranquiçadas formadas de tecido adiposo (PETRACCI; CAVANI, 2012) que se encontram paralelamente às

fibras musculares no peito de aves, podendo acometer os animais em vários níveis, definidos como: normal, moderado ou severo (KUTTAPPAN *et al.,* 2013a). A partir da análise histológica é possível verificar a presença de lesões musculares sobrepostas, como miodegeneração, necrose, infiltração por linfócitos e macrófagos, fibrose, lipidose e outras alterações degenerativas e regenerativas (KUTTAPPAN *et al.,* 2013a; TROCINO *et al.,* 2015).

Ao que parece, quanto maior a severidade de WS, maior o nível de fibrose e a quantidade de adipócitos no músculo. Ao analisar o tecido afetado no microscópio é visível uma grande presença de lise, mineralização e inflamação intersticial ao longo das fibras. As fibras podem apresentar hipereosinofilização, perda das estrias cruzadas e internalização do núcleo, enquanto os músculos de animais saudáveis raramente apresentam esse tipo de alterações. O tecido afetado também tem indícios de fibras fragmentadas e em fase de fagocitose, com tamanhos irregulares, porém existe muita dificuldade em confirmar o tamanho dessas fibras frente à degeneração e regeneração do tecido (KUTTAPPAN *et al.,* 2013a).

Boerboom *et al.* (2014) demonstraram que a manifestação desta patologia está associada a problemas de vascularização no tecido peitoral. Como o músculo desses animais tem um crescimento muito acelerado, o sistema vascular não consegue se desenvolver com a mesma rapidez, levando alguns pontos do músculo à hipóxia. Quando um tecido está sob restrição de oxigênio, a necessidade por ATP aumenta, porém, como a vascularização desses animais é defectível, a oxigenação não é suficiente e os radicais livres não são eliminados de forma eficiente, resultando no estresse oxidativo (LUNDBERG; WEITZBERG, 2008).

Estudos sugerem que a manifestação de WS pode estar associada ao estresse oxidativo, hipóxia localizada e maiores níveis de cálcio intracelular (MUTRYN *et al.,* 2015), e que essas alterações são decorrentes principalmente devido ao peso dos animais, pois quanto mais pesados, maior a propensão de desenvolverem essa miopatia (KUTTAPPAN *et al.,* 2013b).

A qualidade da carne é afetada fundamentalmente por diminuir a quantidade de proteína no músculo e aumentar a quantidade de gordura (KUTTAPPAN *et al.,* 2013a). Também já foi relatado o aumento do pH no músculo peitoral maior de animais afetados com WS (TROCINO *et al.,* 2015). Além da qualidade química, a aceitação dos consumidores em relação aos filés de animais que manifestam a

miopatia é muito baixa, principalmente quando os animais são acometidos pelo grau severo de WS (KUTTAPPAN *et al.,* 2013a).

As lesões causadas por WS não são associadas a agentes infecciosos, e por isso não trazem nenhum risco à saúde do consumidor. Entretanto, mais da metade das pessoas que participaram de um estudo afirmaram que não comprariam filés que apresentassem qualquer grau de estrias brancas (KUTTAPPAN *et al.,* 2012a). Destaca-se também que os comentários negativos foram significativamente maiores para amostras de filés com grau moderado e severo de WS em relação ao grupo normal, o foco principal das críticas era devido à aparência desagradável da carne (KUTTAPPAN *et al.,* 2012a). Como o aspecto da carne só pode ser verificado após o abate dos animais através de análise visual e histológica, esse é um fator limitante para detectar os animais acometidos durante o seu crescimento. Pela aceitabilidade de filés com WS ser muito baixa para consumo *in natura*, esses cortes acabam sendo destinados para subprodutos, perdendo boa parte de seu valor comercial (KUTTAPPAN *et al.,* 2012a).

O peso da ave é um fator essencialmente importante para o desenvolvimento de WS, pois se observou que linhagens com maior capacidade de hipertrofia dos músculos peitorais, foram mais afetadas por essa miopatia (TROCINO *et al.,* 2015). Estudos indicam que animais alimentados *ad libitum* têm menor incidência de WS do que animais submetidos a restrições alimentares (TROCINO *et al.,* 2015).

A prevalência e progressão desse distúrbio se diferenciam em cada país. Ainda não existem informações exatas dos prejuízos acarretados por essa miopatia no Brasil, mas pelo país ser um grande produtor de carne de frango, pode sofrer grande impacto em virtude de WS. Em um estudo realizado com 25 mil frangos em um abatedouro no Sul do Brasil foi relatado que quase 10% destes animais sofriam de algum grau de WS (FERREIRA *et al.,* 2014), o que se assemelha muito aos resultados demonstrados por Petracci *et al.* (2013), que verificaram uma incidência de 12% de WS de graus moderados e severos em animais de linhagens comerciais de crescimento rápido. Já em linhagens de frangos pesados criados em condições experimentais, foram identificados que os índices de WS chegam a 50% considerando os níveis normal, moderado e severo (KUTTAPPAN *et al.,* 2012b).

O desafio intrínseco do melhoramento genético no momento é conseguir reduzir os níveis dessa anomalia sem comprometer o desempenho dos animais, pois

são justamente nos animais de alta produtividade de músculo peitoral que a miopatia WS vem se manifestando mais severamente.

2.4 *WOODEN BREAST*

Outro distúrbio miodegenerativo de grande importância para a avicultura moderna é o WB, popularmente conhecido como peito madeira, que está cada vez mais prevalente nos plantéis e é caracterizado por causar alterações de miodegeneração regenerativa, fibrose e rigidez no músculo peitoral maior de frangos de corte (SIHVO; IMMONEN; PUOLANNE, 2014). Evidências mais recentes apontam que esse distúrbio possa na verdade ser um quadro sistêmico, que desencadeia uma série de alterações fisiológicas e metabólicas no animal (XING *et al*., 2021).

Essa miopatia pode ser detectada a nível microscópico, sendo verificada por uma diminuição das fibras musculares no tecido, uma vez que essas fibras também sofrem de degeneração e se apresentam em tamanhos desuniformes e em uma disposição com arranjo mais frouxo do que o normal. Além disso, também podem ser observadas infiltrações de células inflamatórias na musculatura, assim como pontos de necrose e maiores índices de deposição de colágeno (SIHVO; IMMONEN; PUOLANNE, 2014; SOGLIA *et al*., 2017). Já os sinais que podem ser observados na avaliação macroscópica de WB podem ser entendidos pela rigidez à palpação do músculo, palidez no músculo peitoral, fibrose e excesso de tecido conjuntivo intersticial, e frequentemente essa miopatia vem acompanhada de estrias brancas (SIHVO; IMMONEN; PUOLANNE, 2014).

De acordo com De Almeida Assunção *et al.* (2020), quanto mais severo o nível de WB encontrado nos filés, maiores as mudanças observadas nas características de qualidade e composição química da carne. Entre as principais diferenças observadas, pode-se ressaltar que a concentração de lipídios e colágeno foi superior nos filés com WB, ao tempo que houve uma diminuição no teor de proteína bruta. Os filés amadeirados também apresentaram maior pH do que filés normais, assim como a perda por cozimento foi elevada em relação aos graus da miopatia encontrados.

Xing *et al*. (2021) também relataram que o fígado dos animais afetados por WB apresentavam alterações histopatológicas relacionadas a degeneração

hidrópica (acúmulo de água no meio intracelular), degeneração gordurosa (acúmulo de moléculas lipídicas em células que metabolizam gordura, mas não deveriam armazenar), e níveis aumentados de mieloperoxidase (catalisa a formação de espécies reativas oxidantes). Todas essas discrepâncias em relação ao grupo controle são indícios de lesão hepática, sugerindo que os danos causados pela miopatia WB não se limitam ao músculo peitoral, mas que se estendem a diversos sistemas fisiológicos.

Já no tecido muscular propriamente dito, a miopatia WB causa lesões degenerativas das fibras musculares, que resultam em uma resposta inflamatória do organismo, seguida por um processo de cicatrização que deixa como resquício uma maior deposição de colágeno no tecido (SIHVO; IMMONEN; PUOLANNE, 2014).

Quando os filés afetados com WB são destinados à fabricação de alimentos industrializados, também representam um problema, pois o músculo desses animais acaba sofrendo um processo maior de exsudação, o que dificulta seu processamento (OLIVEIRA *et al*., 2021). Esses são alguns dos motivos pelos quais os cortes afetados com WB são desvalorizados pela indústria, causando grande perda econômica devido a essa miopatia.

A etiologia desse distúrbio ainda não é bem conhecida, porém tem sido associada ao grande desenvolvimento do músculo peitoral dos frangos de corte (KUTTAPPAN *et al.,* 2017). Estudos indicam que a ocorrência de WS e WB estão diretamente relacionadas com o ganho de peso e a idade dos frangos de corte, e muitas vezes essas miopatias são identificadas concomitantemente (KUTTAPPAN *et al.,* 2017).

Até o momento não existem muitos dados verificando se WB afeta o comportamento e bem-estar animal, mas já é caracterizado que animais que apresentam o distúrbio se movimentam menos que animais saudáveis (NORRING *et al*., 2019). Presume-se que os casos mais severos da miopatia possam estar causando desconforto nos animais, mas o maior tempo de repouso também pode ser atribuído ao fato de que a maior parte dos casos de WB se apresentam nos animais mais pesados (NORRING *et al*., 2019).

2.5 GENÉTICA E AS MIOPATIAS PEITORAIS

Ao comparar a manifestação de WS em duas linhagens, uma normal e uma selecionada para alto desempenho do músculo peitoral, Trocino *et al.* (2015) constataram a prevalência de 19,5% de animais acometidos pelo grau severo da miopatia nos animais de grande desempenho, enquanto os animais do genótipo padrão tiveram incidência de apenas 9,5%. Hoving-Bolink *et al.* (2000) e Joiner *et al.* (2014) sugerem que frangos com alto rendimento do músculo peitoral e crescimento rápido acabam tendo menor densidade de vasos capilares no músculo e, por consequência, têm sua disponibilidade de oxigênio reduzida, podendo contribuir com o aumento da incidência de miopatias.

Uma série de genes e *loci* que controlam características quantitativas (QTL, do inglês *Quantitative trait loci*) estão envolvidos na regulação do rendimento do músculo peitoral de aves, e também já foi verificada a associação de 42 polimorfismos de base única (SNPs) em relação às características que conferem a qualidade da carne e WS (PAMPOUILLE *et al.,* 2018). Isso demonstra que é muito complexo definir exatamente qual seria o gatilho para a manifestação das miopatias peitorais.

Estimativas de herdabilidade para WS variam de 0,19 a 0,34, mesmo que essa condição seja muito mais prevalente em linhagens pesadas de frango, enquanto a correlação genética entre peso corporal e rendimento do músculo do peito foi estimada como baixa a moderada (BAILEY *et al.,* 2015). Entretanto, Alnahhas *et al.* (2016) destacaram a genética como um dos fatores principais para que os animais desenvolvessem WS, com um forte determinismo genético (h2 = 0,65 ± 0,08). Em relação ao pH muscular, linhagens selecionadas para maiores níveis de pH muscular foram significativamente mais afetadas do que linhagens selecionadas para baixo pH muscular, o que está intimamente ligado à capacidade do músculo em armazenar energia como carboidrato. A miopatia WS também foi correlacionada geneticamente com a quantidade de gordura intramuscular do músculo peitoral maior dessas aves (rg 0,64 ± 0,09) (ALNAHHAS *et al*., 2016).

Pampouille *et al.* (2018) identificam alguns genes candidatos envolvidos no metabolismo muscular de aves que podem estar associados à manifestação de WS: *MYH15*, *MYH1E*, *MYH1B*, *MYH1F*, *MYH13* e *MYOCD*, que são genes associados à função de regeneração e reparo das fibras musculares; *PDGFRα*, associado à

adipose e a fibrose; *COL6A3*, *FN1* e *SGCB*, relacionados à matriz extracelular e a composição do sarcolema; *PNPLA*, um gene relacionado ao metabolismo muscular e os genes *FN1*, *COL6A3*, *SGCB*, *LRSAM*, que são conhecidos por suas funções em distúrbios neuromusculares humanos. Assim, acredita-se que essa miopatia tenha origem poligênica complexa e que estudos adicionais são necessários para definir especificamente quais genes atuam para que frangos de corte desenvolvam essa patologia.

A expressão de alguns genes como *TGFβ1* e *CTGF* tem sido correlacionada positivamente com a presença e gravidade da desregulação fisiológica de WS. Esses genes estão envolvidos com o desenvolvimento do tecido conjuntivo, assim como os genes *TGFβ1* e *PPARG* têm demonstrado estarem correlacionados com a indução de fibrose e adipose nos músculos de aves, e consequentemente teriam relação com WS (PRAUD *et al.,* 2020).

Em estudos de transcriptômica, foram identificados 1441 genes expressos diferencialmente (DE) no PMM de frangos normais e afetadas com WS aos 42 dias de idade, sendo 772 regulados positivamente e 669 regulados negativamente em relação ao grupo normal (MARCHESI *et al*., 2019). Nesse trabalho, os 10 genes com maior expressão e os 10 genes com menor expressão no grupo afetado com WS em relação ao controle, se destacaram por ter funções relacionadas à fisiologia muscular, angiogênese e inflamação. Já na análise do conjunto de todos os 1441 genes DE obtidos, foram identificados todos os processos em que eles estavam evolvidos, entre eles estão: mecanismos de angiogênese, estrutura e desenvolvimento de tecidos, hipóxia localizada, estresse oxidativo, inflamação, resposta à lesão muscular, metabolismo de carboidratos e aumento do cálcio intracelular. O perfil do transcriptoma diferencial entre o grupo normal e afetado por WS poderia justificar as alterações fenotípicas, fisiológicas e histológicas causadas pela miopatia WS (MARCHESI *et al.,* 2019). Além disso, os genes *CA2*, *CSRP3*, *MYLK2*, *CALM2*, *PLIN1* e *DNASE1L3* se apresentaram diferencialmente expressos entre frangos normais e afetados com WS aos 42 dias (MARCIANO *et al*., 2021).

Estudos sobre a miopatia WB identificaram 51 SNPs distribuídos em 11 cromossomos do genoma de *Gallus gallus* associados ao desenvolvimento dessa condição (LAKE; DEKKERS; ABASHT, 2021). Os principais genes candidatos para o desenvolvimento do peito madeira foram *CDKN1C*, *CTSD*, *KCNQ1*, *LSP1*, *SLC22A18*, *USH1*, *USH1C* e *DNM2* e *GGA30*, sendo que parte deles tem função

relacionada a alterações no metabolismo da insulina (LAKE; DEKKERS; ABASHT, 2021).

Também é evidenciado que o perfil de expressão gênica em animais com peito madeira se modifica em relação à idade dos frangos. Com duas semanas os animais afetados por WB apresentavam 41 genes diferencialmente expressos (DE) em relação ao grupo normal, essa diferença aumentou para 618 genes DE entre os grupos em animais de 4 semanas (PAPAH *et al*., 2018). Em animais afetados com WB de duas semanas os principais processos biológicos regulados pelos genes DE estavam relacionados à diferenciação do músculo esquelético, resposta ao estresse, resposta à inflamação e estresse oxidativo, no entanto, com quatro semanas os processos regulados pelos genes DE estavam mais voltados à adesão celular, ligação de ATP e vias metabólicas (PAPAH *et al*., 2018).

Existem indícios que animais afetados por WB possuem desregulações gênicas relacionadas ao metabolismo de colágeno e fibrose no músculo peitoral, sendo que genes chaves identificados em frangos com peito madeira foram *COL4A1, LAMA4, COL4A2, FBLN5, FBN1, COL15A1, RRC32, PLXDC2, LAMA2* e *LAMC1* (BORDINI *et al*., 2021).

Uma série de genes envolvidos com doenças vasculares foram regulados positivamente em animais com WB, entre eles *C3, C7*, *C1R*, *C1S*, *C1QA*, *C1QB*, *C1QC* e *C3AR1*, assim como genes associados à resposta inflamatória, incluindo *PTGS2*, *CSF-1* e *CSF-1R* (PAPAH *et al*., 2018).

Para distúrbio WB baixas herdabilidades (h^2 = 0,10) têm sido estimadas (BAILEY *et al*., 2015). Porém, altas herdabilidades para diversos metabólitos circulatórios envolvidos no peito madeira foram estimadas, como por exemplo, 1-metilhistidina (h2= 0,57 ± 0,2), palmitol dihidroesfingomielina (h^2= 0,77 ± 0,17), C-glicosiltriptofano (h2= 0,89 ± 0,15), entre muitos outros metabólitos envolvidos no metabolismo da histidina e esfingolipídios (LAKE *et al*., 2022).

Além disso, Zambonelli *et al*. (2016), analisando o perfil de expressão gênica em animais afetados pelo distúrbio do peito madeira, identificaram intensa atividade de genes que atuam no metabolismo de glicose, lipidose, fibrose, hipóxia e estresse oxidativo no músculo desses animais. Shu *et al*. (2021) também evidenciaram genes alvo importantes para miRNAs em animais com WB relacionados ao metabolismo hipóxico, metabolismo fibrótico e de colágeno e metabolismo glicolítico.

2.6 MICRORNAS E AS MIOPATIAS PEITORAIS

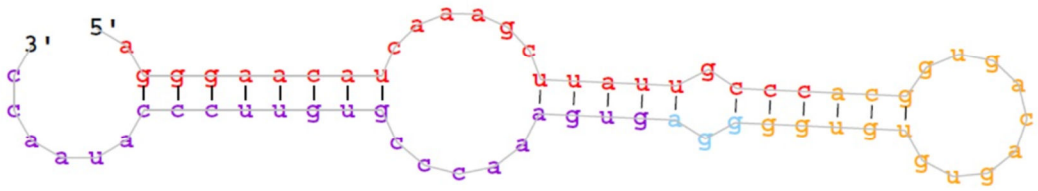
Atualmente, sabe-se que grande parte da variação fenotípica entre indivíduos não é explicada apenas com a genética mendeliana, que abrange os mecanismos baseados na modificação de sequências do DNA (MISKA; FERGUSON-SMITH, 2016). Existem diversas modificações químicas regulando a expressão gênica que conferem funções específicas para cada tipo de célula, e guiando respostas celulares ao ambiente. Esses são ditos mecanismos epigenéticos (SKVORTSOVA; IOVINO; BOGDANOVIĆ, 2018).

Ainda não está totalmente esclarecido como ocorrem estas respostas ambientais através dos mecanismos epigenéticos, mas as modificações químicas mais conhecidas por regularem a expressão gênica são: a metilação do DNA, as modificações das histonas e os microRNAs (miRNAs) (SKVORTSOVA; IOVINO; BOGDANOVIĆ, 2018). É importante destacar que esses mecanismos epigenéticos também são transmitidos para as próximas gerações, então uma resposta a algum estresse como calor, restrição alimentar entre outros, pode se manifestar mesmo em descendentes que nunca foram submetidos a esse estresse (SKVORTSOVA; IOVINO; BOGDANOVIĆ, 2018).

Alguns estudos demonstram que a transmissão dos estados epigenéticos, tanto paternos quanto maternos foram igualmente eficientes (RASSOULZADEGAN, *et al*., 2006). Os miRNAs foram relatados pela primeira vez na década de 90 em estudos com *Caenorhabditis elegans*, como sendo pequenos RNAs não codificantes (LEE; FEINBAUM; AMBROS, 1993; WIGHTMAN; RUVKUN, 1993), e posteriormente diversos estudos comprovaram que tais moléculas participam de um mecanismo de silenciamento de genes presente em animais, plantas, vírus e fungos.

Os miRNAs são pequenas moléculas de RNA, normalmente com 17 a 25 nucleotídeos, com capacidade para reconhecer o RNA mensageiro e assim, afetar a expressão dos genes (**Figura 2**). Estes existem naturalmente no genoma dos animais e de outros organismos.

Figura 2 - *Estrutura de um miRNA* primário



Fonte: miRDeep2.

Fire *et al*. (1998) descobriram que um RNA fita-dupla (dsRNA) é o gatilho para o silenciamento e que, esse RNA se torna dupla fita a partir de uma dobra sobre si mesmo, como uma espécie de grampo. Os dsRNAs se diferenciam em pequenos RNAs de interferência (siRNA), microRNAs (miRNAs) e outros, que atuam em sequências alvo específicas para que aquele gene seja silenciado (ZAMORE *et al*., 2000). Tuschl *et al*. (1999) identificaram que esse silenciamento acontece por um tipo de indução à degradação do RNA mensageiro (mRNA) alvo, antes ou durante a tradução, impedindo que a proteína seja traduzida e funcional. O que acontece é que uma vez que o dsRNA dá origem às moléculas de RNA de interferência, elas são capazes de se ligar à uma proteína Argonauta, e juntos formam um complexo (RISC) de silenciamento gênico pós-transcricional que catalisa a clivagem do mRNA alvo (HUTVAGNER; ZAMORE, 2002; MOURELATOS *et al*., 2002).

Os miRNAs são transcritos do DNA pela RNA polimerase II e o resultado disso é um miRNA primário (pri-miRNA), que é fita dupla e está em formato de grampo sobre si mesmo. Posteriormente esses fragmentos são processados pela enzima RNase III Drosha para formar um microRNA precursor (pre-miRNA), onde um lado do grampo é processado e sofre clivagem, então os pre-miRNAs são exportados do núcleo pela exportina. Quando chegam no citoplasma, essas moléculas intermediárias são processadas pela enzima Dicer, onde o outro lado do grampo é processado e a molécula se transforma em um miRNA maduro. Os miRNAs maduros podem se ligar à proteína Argonauta e formar o complexo RISC de silenciamento gênico. Os miRNAs se ligam ao mRNA através do emparelhamento de bases no local alvo e induzem a sua clivagem (BARTEL, 2009). Com esse mecanismo, os miRNAs regulam a expressão gênica. Os miRNAs são altamente conservados entre as espécies devido a região chamada de “semente”, que são alguns nucleotídeos que conferem especificidade para o miRNA (CALIN *et al*., 2007).

Os miRNAs têm um papel muito importante no desenvolvimento embrionário dos animais, permitindo que vários mecanismos se expressem neste momento para a formação dos tecidos do novo indivíduo, mecanismos que depois são silenciados durante a vida adulta do organismo (TANG *et al*., 2007; LIU *et al*., 2012). Adaptações fisiológicas nesse mecanismo são resultantes de fatores ambientais e estresses aos quais os organismos são submetidos ao longo da vida, sendo que o perfil de expressão de miRNAs também é transmitido para as novas gerações (GAPP *et al*., 2014; RODGERS *et al*., 2015).

Os miRNAs estão envolvidos em quase todos os processos celulares nos animais, atuando em processos importantes para o desenvolvimento, diferenciação celular e homeostase (GEBERT; MACRAE, 2019). Atuações desse mecanismo já são relacionadas a diversas doenças, inclusive as miopatias peitorais em frangos de corte (SHU *et al*., 2021). Vários miRNAs foram encontrados diferencialmente expressos entre animais com WB em relação a animais com ausência desta miopatia. Entre eles, os miRNAs gga-miR-146-5p, gga-miR-29, gga-miR-21-5p, gga-miR-133a-3p e gga-miR-133b parecem ter um efeito importante sobre o peito madeira (SHU *et al*., 2021).

A atividade dos miRNAs pode regular várias vias fisiológicas nos animais, mas ainda não existem estudos verificando se o perfil de expressão de miRNAs é um fator determinante para diferenciar as miopatias peitorais *White Striping* e *Wooden Breast* em frangos de corte aos 28 dias de idade.

2.7 BIOINFORMÁTICA

As análises de miRNAs utilizam diversos aplicativos de bioinformática e Bancos de Dados, das quais, mais de 1000 ferramentas com aplicações nesse tipo de análise já foram documentadas desde 2003 (CHEN *et al*., 2019c). Os programas de bioinformática incluem informações sobre a sequência e anotação dos miRNAs, predição de genes alvos, identificação de novos miRNAs e análises de perfil de expressão de miRNAs. A base de dados de miRNAs mais utilizada atualmente é o miRBase (KOZOMARA; GRIFFITHS-JONES, 2014).

A quantidade de dados gerados de miRNAS em um projeto pode chegar a milhões de sequências e, por isso, a manipulação dos dados de sequenciamento

exige ferramentas específicas, conhecimento em linguagem de programação e *scripts* em sistema operacional Linux.

O primeiro passo na análise dos dados brutos do sequenciamento é o controle de qualidade. Os arquivos FASTQ são filtrados de acordo com o tipo de experimento e os parâmetros podem ser definidos pelos autores. Para garantir a qualidade nos resultdos do sequenciamento de miRNAs é recomendado a remoção de fragmentos com baixa qualidade média Phred (PHRED>=20 por janela de 5 bases) e que contenham bases ambíguas, isto é, identificadas com a letra “N”. Como os miRNAs têm aproximadamente de 18 a 22 nucleotídeos (nt), as leituras também podem ser filtradas por tamanho. Um programa muito utilizado para essas filtragens é o Trimmomatic (BOLGER, 2014) que permite a configuração de vários parâmetros, podendo se adequar a uma grande variedade de análises genéticas.

Um problema que ocorre na etapa anterior ao sequenciamento é a geração de artefatos de PCR, que produzirá várias cópias do mesmo fragmento a ser sequenciado podendo interferir nos resultados. Para resolver este problema, uma alternativa é a adição de “código de barras” de oligonucleotídeos chamados de Identificadores Moleculares Únicos (UMI). Estes UMIs se baseiam em uma sequência aleatória que é diferente para cada fragmento e que durante a etapa de PCR esse “código de barras” é amplificado junto com o miRNA. Após o sequenciamento, por meio da sequência UMI, é possível identificar se as cópias do miRNA são reais ou erros provenientes da amplificação por PCR (SMITH; HEGER; SUDBERY, 2017). A interpretação dos UMIs é feita pelo software Umi Tools, sendo responsável por identificar e remover as duplicatas de PCR (SMITH; HEGER; SUDBERY, 2017).

Com as sequências com erros de artefatos de PCR removidas, as sequências restantes serão manipuladas pelo programa miRDeep2 (FRIEDLÄNDER *et al*., 2012), que foi desenvolvido para analisar dados de miRNAs e contém informações de várias espécies. Nessa etapa, o programa é capaz de identificar miRNAs já disponíveis nas bases de dados, assim como inferir novos miRNAs a partir de um algoritmo que analisa os sítios de ligação, a energia de troca disponível, as sequências precursoras, previsão de conformação de estruturas secundárias e outras características importantes para que um miRNA seja funcional (FRIEDLÄNDER *et al*., 2012).

Posteriormente à identificação dos miRNAs, é realizado o mapeamento dos mesmos contra o genoma da espécie de referência, em nosso caso, o de *Gallus gallus*, e então é feita a contagem dos miRNAs. Uma vez tendo o número de cópias dos miRNA em cada amostra, o próximo passo é realizar a expressão diferencial dos miRNAs entre os grupos de amostras. Para esta etapa, foi utilizado o programa edgeR (ROBINSON; MCCARTHY; SMYTH, 2010) sendo um pacote de análises estatísticas desenvolvido em linguagem R que fornece todos os recursos para a análise de expressão diferencial.

Para entendermos qual o real potencial de atuação de um miRNA, a etapa seguinte deve ser a de predição de alvos. Devido aos miRNAs degradarem mais de um tipo de RNA mensageiro (GEBERT; MACRAE, 2019), a predição de alvos busca todos os mRNAs que podem sofrer a ação dos miRNAs. Essa previsão se baseia de maneira geral na complementariedade das bases entre o miRNA e o mRNA, que pode acontecer de maneira canônica, referindo-se ao alinhamento com a região de *seed* que contém de 6 a 8 nucleotídeos, ou de maneira não canônica, que é quando o miRNA tem um pareamento para além da região de *seed* (FEITOSA *et al*., 2022).

Já existem diversas ferramentas especializadas na predição de alvos de miRNA, cada uma com suas vantagens e suas limitações, sendo altamente recomendável a associação de mais de uma ferramenta de predição nas análises. Assim, os resultados que forem comuns para mais de uma ferramenta apresentarão maior precisão e confiabilidade. A plataforma mais usada para predição é o TargetScan, porém ele analisa cada miRNA separadamente, dificultando a análise de listas longas de miRNAs. Uma ferramenta que se adequa bem a grandes quantidades de dados de miRNA é o sRNAtoolbox (RUEDA *et al*., 2015), que é uma plataforma online robusta que se propõem a fornecer diversas ferramentas de análises de pequenos RNAs, tanto para animais quanto para plantas. Ela integra 4 ferramentas de predição de alvos de miRNAs em animais em uma plataforma só, sendo elas: *Pita, miRanda, TargetSpy* e *Simple Seed Analysis*.

Posteriormente à predição dos genes alvo dos miRNAs diferencialmente expressos, o enriquecimento funcional e a ontologia gênica trazem os dados da potencial atuação biológica dos miRNAs. Essas análises podem ser feitas através da ferramenta online ShinyGO (GE; JUNG; YAO, 2020), que se baseia em um grande banco de dados para analisar uma lista de genes e identificar as vias metabólicas em que eles estão presentes. A plataforma ShinyGO possui recursos para

visualização gráfica dos resultados e também direciona o resultado do enriquecimento para o banco de dados KEGG, que contempla uma vasta coleção de informações sobre as vias metabólicas e interações moleculares das vias nos organismos.

Finalizados todos esses processos, inicia-se a fase de investigação da atuação dos miRNAs em uma determinada situação, ou mesmo comparar o perfil de miRNAs entre grupos experimentais, assim como as possíveis alterações que podem estar ligadas à atividade desses pequenos RNAs.

3 ARTIGO: DIFFERENTIAL EXPRESSION OF MIRNAS INVOLVED WITH PECTORAL MYOPATHIES IN YOUNG BROILERS: INSIGHTS FROM A COMPARATIVE TRANSCRIPTOME ANALYSIS

Os resultados desta dissertação são apresentados na forma de artigo submetido, com as seções de acordo com as orientações da Revista BMC Genomics.

Differential expression of miRNAs involved with pectoral myopathies in young broilers: insights from a comparative transcriptome analysis

Mariane Spudeit Dal Pizzol[1], Adriana Mércia Guaratini Ibelli[2,3], Maurício Egídio Cantão[2], Francelly Geralda Campos[4], Haniel Cedraz de Oliveira[4], Jane de Oliveira Peixoto[2,3], Lana Teixeira Fernandes[2], Fernando de Castro Tavernari[1,2], Marcos Antônio Zanella Morés[2], Ana Paula Almeida Bastos[2,3], Mônica Corrêa Ledur[1,2].

Mariane Spudeit Dal Pizzol1
E-mail: mari_spudeit@hotmail.com

Adriana Mércia Guaratini Ibelli2,3
E-mail: adriana.ibelli@embrapa.br

Mauricio Egídio Cantão2
E-mail: mauricio.cantao@embrapa.br

Francelly Geralda Campos4
E-mail: francelly.campos@ufv.br

Haniel Cedraz de Oliveira4
E-mail: hanielcedraz@gmail.com

Jane de Oliveira Peixoto2,3
E-mail: jane.peixoto@embrapa.br

Lana Teixeira Fernandes2

E-mail: lanatf@yahoo.fr

Fernando de Castro Tavernari1,2
E-mail: fernando.tavernari@embrapa.br

Marcos Antônio Zanella Mores2
E-mail: marcos.mores@embrapa.br

Ana Paula Almeida Bastos2,3
E-mail: ana.bastos@embrapa.br

Mônica Corrêa Ledur1,2*
E-mail: monica.ledur@embrapa.br

*Corresponding author
Mônica Corrêa Ledur
E-mail: monica.ledur@embrapa.br
Embrapa Suínos e Aves, Rodovia BR-153, Km 110, Distrito de Tamanduá, Caixa Postal: 321, Concórdia, Santa Catarina 89715-899, Brazil.

3.1 ABSTRACT

Background: White Striping (WS) and Wooden Breast (WB) pectoral myopathies are relevant disorders for modern broiler production worldwide. Several studies aimed to elucidate the genetic components involved with the occurrence of these myopathies. However, epigenetic factors that trigger or differentiate these two conditions are still unclear. The aim of this study was to identify miRNAs differentially expressed (DE) between normal and WS and WB-affected broilers, and to verify the possible role of these miRNAs in metabolic pathways related to the manifestation of these pectoral myopathies in 28-day-old broilers.

Results: Five miRNAs were DE in the WS vs control (gga-miR-375, gga-miR-200b-3p, gga-miR-429-3p, gga-miR-1769-5p, gga-miR-200a-3p), 82 between WB vs

control and 62 between WB vs WS. Several known miRNAs were associated with WB, such as gga-miR-155, gga-miR-146b, gga-miR-222, gga-miR-146-5p, gga-miR-29, gga-miR-21-5p, gga-miR-133a-3p and gga-miR-133b. Most of them had not previously been associated with the development of this myopathy in broilers. We have also predicted 17 new miRNAs expressed in the broilers pectoral muscle. DE miRNA target gene ontology analysis enriched 6 common pathways for WS and WB compared to control: autophagy, insulin signaling, FoxO signaling, cell cycle, endocytosis, and metabolic pathways. The WS vs control contrast had two unique pathways, ERBB signaling and the *mTOR* signaling, while WB vs control had 14 unique pathways, with ubiquitin-mediated proteolysis and endoplasmic reticulum protein processing being the most significant.

Conclusions: We found miRNAs DE between normal broilers and those affected with breast myopathies at 28 days of age. Our results also provide novel evidence that miRNAs are involved in the regulation of WS and in the differentiation of both WS and WB myopathies. Moreover, WB-affected broilers might be more susceptible to the miRNA's regulation. The DE miRNA predicted-target genes reveled important pathways involved with the two breast myopathies. Overall, our study provides insights into miRNAs and pathways involved with the occurrence of WS and WB helping to better understand these chicken growth disturbs in an early age.

Keywords: epigenetics, wooden breast, White striping, small RNAs, chickens.

3.2 BACKGROUND

Science and technology have led to a significant increase in poultry chain productivity in recent decades (Zuidhof *et al*., 2014). However, these advancements have been related to the onset of some physiological problems in broiler chickens (L. R. Chen *et al*., 2019; Julian, 1998; Kuttappan *et al*., 2012). The main pathological changes reported have been abnormalities in the chicken muscle tissues, which develop during the growth phase and progressively worsen during the productive life of the animal (Soglia *et al*., 2021). Currently, two main problems affecting broilers are the degenerative disorders caused by White Striping (WS) and Wooden Breast (WB) pectoral myopathies (Ferreira *et al*., 2014; Praud *et al*., 2020)

The main feature of WS myopathy is the presence of white stripes that form parallel to muscle fibers on the breast of affected animals (Kuttappan *et al*., 2013; Petracci and Cavani, 2012). These stripes are mainly composed by adipose tissue, and histological analysis reveals the presence of overlaid muscle lesions such as myodegeneration, necrosis, lymphocyte and macrophage infiltration, fibrosis, lipidosis, and other degenerative changes (Kuttappan *et al*., 2013; Trocino *et al*., 2015). On the other hand, WB myopathy is characterized by regenerative myodegeneration, fibrosis and pectoral muscle hardness (Sihvo *et al*., 2014). WB also causes several microscopic changes, such as irregular and disarranged fibers, infiltration of inflammatory cells, increased collagen deposition in the tissue, and is often accompanied by WS (Sihvo *et al*., 2014; Soglia *et al*., 2019).

Both WS and WB disorders do not represent a risk to the consumer´s health; however, they negatively affect the physicochemical characteristics of the meat (Mudalal *et al*., 2014; Sihvo *et al*., 2014; Trocino *et al*., 2015). Moreover, fillets affected by myopathies tend to be rejected by consumers (Kuttappan *et al*., 2013). These are some of the reasons why the cuts of the affected animals are undervalued and ultimately designated for by-products in the industry. Meat from affected chickens also represents problems during processing, as their muscle are more exudative, in addition to the large deposition of collagen which significantly impact the texture of the food. Therefore, product correction is needed through industry interventions (de Oliveira *et al*., 2021; Sanchez Brambila *et al*., 2016).

The myopathic pectoral muscle causes damage to the entire poultry chain, both due to their low yield caused by cooking and dripping losses and their devalued cuts (Kuttappan *et al*., 2016). Carcass condemnation rates caused by myopathies are reported to be close to 0.8%, preventing the sale of the whole chicken (which has high commercial value) and resulting in estimated economic losses by approximately BRL 5,90 (US$ 1.20) per kilogram of meat, and daily losses of up to BRL 21,800.00 (US$ 4.300,00) in a slaughterhouse in Brazil (Zanetti *et al*., 2018).

Genetics has been considered an important factor for the development of WS and WB in broilers, with moderate to high heritability for WS ($h^2 = 0.18 \pm 0.01$ to $h^2 = 0.65 \pm 0.08$) (Alnahhas *et al*., 2016; Bailey *et al*., 2015) and low heritability for WB ($h^2 = 0.10$) (Bailey *et al*., 2015). Differences in the occurrence of myopathies were found among fast-growing commercial lines (Gratta *et al*., 2019; Livingston *et al*., 2019).

Several authors have reported that high-breast-yielding broilers are more affected by myopathies than standard broiler lines (Alnahhas *et al*., 2016; Bailey *et al*., 2015; Bordini *et al*., 2022; Lorenzi *et al*., 2014; Pampouille *et al*., 2018; Petracci *et al*., 2013; Praud *et al*., 2020; Trocino *et al*., 2015).

Transcriptomic analyses of the pectoralis major muscle have provided the identification of the messenger RNA (mRNA) expression profile in broilers affected by myopathies (Bordini *et al*., 2022; Brothers *et al*., 2019; Marchesi *et al*., 2018; Marciano *et al*., 2021; Mutryn *et al*., 2015; Zambonelli *et al*., 2016). These functional studies have pointed out several candidate genes for the development of these disorders. However, the contribution of epigenetic factors to the development of breast myopathies are still a challenge. Nevertheless, a study has already associated miRNAs profile with the manifestation of WB myopathy (Shu *et al*., 2021).

Given the significance of miRNAs in muscle development and their potential role in the regulation of myopathies in other species (Singh *et al*., 2020; Wang *et al*., 2018; Williams *et al*., 2009), this study aims to identify differences in the expression profile of miRNAs between normal broilers and those affected by WS and WB. Additionally, this study seeks to evaluate the potential of miRNAs to act on metabolic pathways associated with the onset and differentiation of pectoral myopathies in 28-day-old broiler chickens.

3.3 RESULTS

3.3.1 Pathological findings

From the 30 pectoralis major muscle (PMM) evaluated, it was possible to classify 27 of them: 4 as normal (no apparent macroscopic lesions), 16 with WS and 7 with WB, according to the classification criteria established by Kuttappan *et al*. (2013) and Sihvo, Immonen and Puolanne (2014) (**Figure 3**).

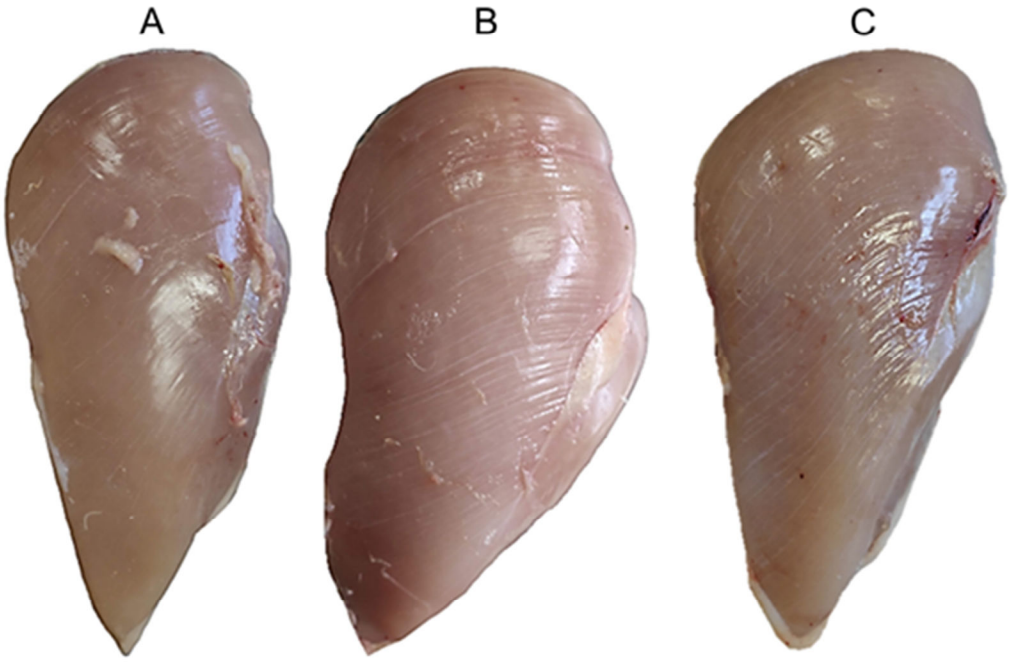


Figure 3 *- Breasts from 28-day-old broilers representing the macroscopic evaluation of the normal (control) (A), white striping (B) and wooden breast (C) groups.*

The histopathological evaluation of 27 out of 30 initial samples revealed 4 normal muscle samples showing organized muscle fibers of regular size with rare hypereosinophilic fibers (**Figure 4A**). Sixteen (16) samples showed lesions consistent with WS: mild to moderate presence of hypereosinophilic fibers, moderate number of degenerated and necrotic fibers, an increase in the spaces between fibers and muscle bundles and moderate proliferation of intramuscular adipocytes (**Figure 4B**). Finally, seven samples presented WB compatible lesions: high number of hypereosinophilic and necrotic muscle fibers, moderate to high proliferation of fibroblasts, muscle fibers showing different sizes, looser cells arrangement with significant increase in the spaces between fibers and muscle bundles, presence of interstitial connective tissue, mild heterophile infiltration and moderate intramuscular adipose tissue (**Figure 4C**).

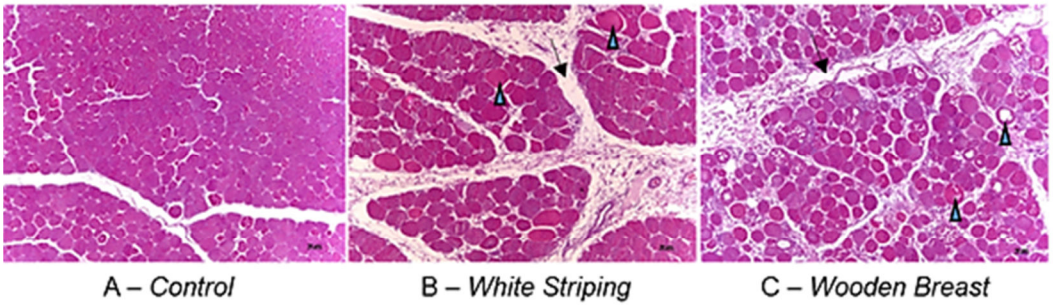


Figure 4 *- Histopathological analysis of 28-day-old chicken breasts showing microscopic features of the control (A), white striping (B), and wooden breast (C) groups. Increase in space between muscle bundles (arrow), several degrees of degenerated fibers (arrowhead).*

Based on the macroscopic and microscopic analyses, the muscle samples were classified into three groups: control (none or slight lesions), WS-affected, and WB-affected groups. For miRNA analysis, the most representative samples of each group were selected: three samples for the control group, five samples for the WS-affected group, and six samples for the WB-affected group.

3.3.2 Sequencing, quality control and mapping

Approximately 205 million reads were sequenced across all samples, resulting in an average of 15.7 million reads per sample. After sequences quality control, a mean of 7.5 million reads per sample remained, which were aligned against the ribosomal (rRNA) and transporter RNAs (tRNA) using RFAM database realease 14. Around 1.1% of those sequences were removed for downstream analysis. Then, an average of 67.5% of the sequences were mapped in the Gallus gallus genome (**Supplementary File 1 - Table S1**).

3.3.3 miRNA identification and differential expression analysis

A total of 844 miRNAs were detected based on all miRNAs identified by miRDeep2. From those, 755 were known miRNAs and 89 were firstly described in this study (**Figure 5**). After filtering the lowly expressed reads using "filterbyexpr"

function from EdgeR (Robinson *et al.*, 2010), 303 miRNAs were determined as expressed, including 286 known miRNAs and 17 new ones.

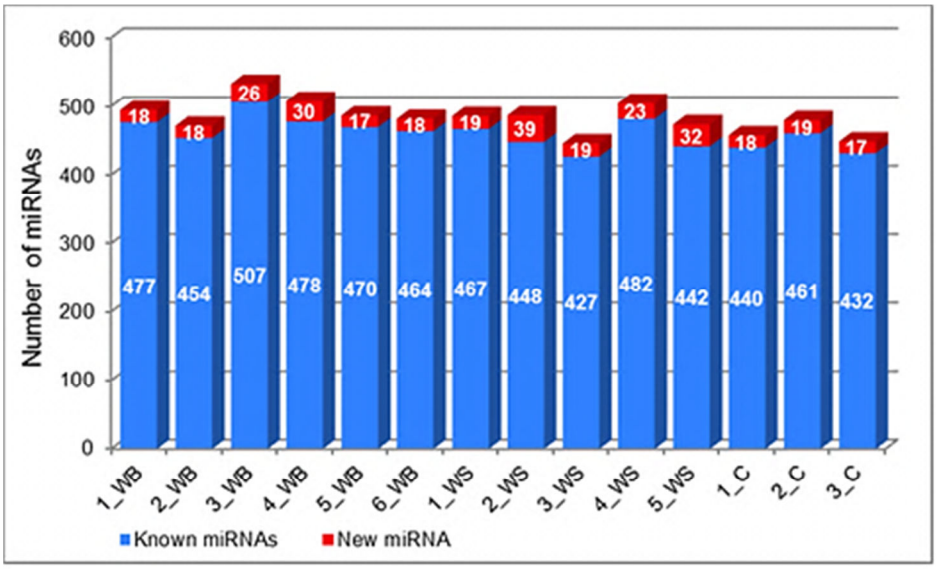


Figure 5 *- Number of identified (known and new) miRNAs in each sample of pectoralis muscle tissue.*

A multidimensional scaling plot (MDS) was generated based on the profile of expressed miRNAs, and the three groups were separated according to their respective physiological conditions (**Figure 6A**), showing a consistent miRNA profile in the samples within each group, indicating homogeneity. Similar separation pattern was also observed in the heatmap (**Figure 6B**).

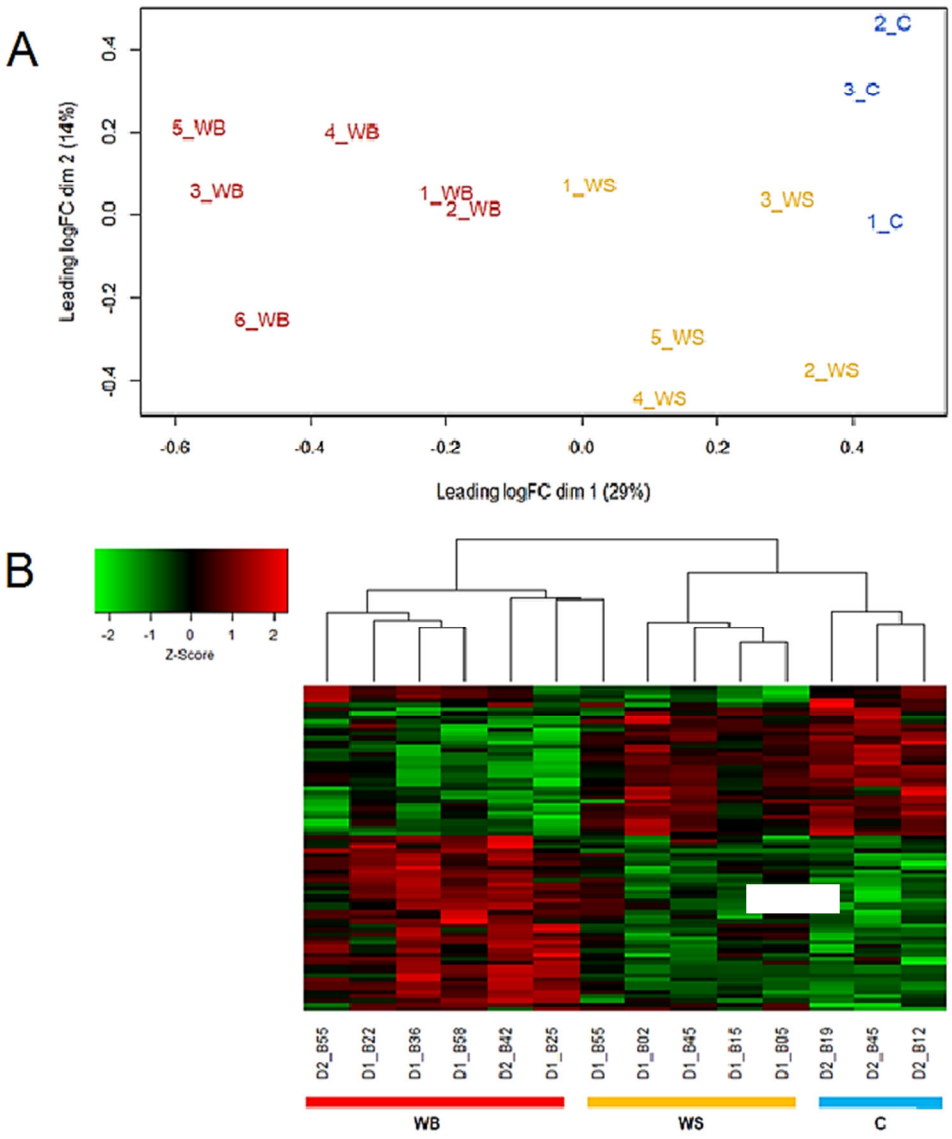


Figure 6 - *Multidimensional scale (MDS) plot (A) and heatmap (B) showing the separation of control, white striping (WS) and wooden breast (WB) groups through the miRNA's expression profile. Heatmap hierarchically grouping the expression of 80 DE miRNAs that most di*

For the DE analysis, three comparisons were performed: WS vs control group, WB vs control group and WB vs WS group. Considering WS vs control, five miRNAs were DE, four downregulated and one upregulated in the WS group (**Table 1**).

Table 1 *- Differentially expressed miRNAs between 28-day-old control and white striping-affected broilers.*

miRNAs	logFC	logCPM	p-value	FDR
gga-miR-375	-4.48	3.51	7.22E-06	0.0022
gga-miR-200b-3p	-2.90	4.84	0.00021	0.0230
gga-miR-429-3p	-2.56	4.25	0.00023	0.0230
gga-miR-1769-5p	3.67	0.37	0.00057	0.0435
gga-miR-200a-3p	-2.74	7.17	0.00074	0.0451

logFC: log fold-change; logCPM: log copy per million; FDR: false discovery rate

When comparing WB and control groups, 82 miRNAs were DE, 43 were upregulated and 39 were downregulated in the WB-affected group (Table 2, Supplementary file 1 – Table S2).

Table 2 *- Top 5 up and downregulated miRNAs in the WB-affected compared to the control group.*

	miRNA	logFC	logCPM	PValue	FDR
Upregulated	chr22_10817	5.51	-0.29	0.0014	0.0089
	gga-miR-1769-5p	3.69	0.36	0.0003	0.0033
	gga-miR-3530-3p	3.68	0.69	0.0001	0.0013
	gga-miR-205a	3.30	3.51	0.0009	0.0064
	gga-miR-222b-5p	2.60	1.34	8.26E-07	4.17E-05
Downregulated	gga-miR-6553-5p	-1.79	1.21	0.0058	0.0274
	gga-miR-6553-3p	-1.87	3.97	0.0013	0.0086
	chr2_9820	-2.00	3.83	2.79E-07	2.11E-05
	chr2_9097	-2.72	0.78	0.0004	0.0033
	gga-miR-122-5p	-4.42	9.15	0.0066	0.0294

Considering the comparisons of the two affected groups WB with WS, 61 miRNAs were DE, 37 upregulated and 24 downregulated in the WB group (**Table 3, Supplementary file 1 – Table S3**).

Table 3 *- Top 5 up and downregulated miRNAs in the WB compared to WS-affected group.*

	miRNA*	logFC	logCPM	PValue	FDR
Upregulated	chr22_10817	5,51	-0,29	3,45E-05	0,000826
	gga-miR-1663-5p	4,72	0,14	3,89E-09	1,18E-06
	gga-miR-200b-3p	3,25	4,83	7,68E-06	0,000258
	gga-miR-375	3,21	3,50	0,000298	0,004297
	gga-miR-200a-3p	3,12	7,17	3,55E-05	0,000826
Downregulated	gga-miR-144-3p	-1,20	5,34	0,0016	0,016015
	gga-miR-193a-3p	-1,24	3,65	0,0004	0,0061
	gga-miR-451	-1,32	9,25	2,41E-05	0,000724
	chr2_9820	-1,41	3,83	5,48E-05	0,001186
	chr2_9097	-1,89	0,78	0,007947	0,043001

* miRNA names starting with “chr” are predicted for the first time in this study.

Evaluating the three contrasts (**Figure 7**), it was possible to observe in the Venn diagram that 31 miRNAs were exclusively DE between WB-affected and the control group. Additionally, 7 miRNAs were DE only in the contrast between WB and WS. No miRNA was exclusively DE in the WS vs control group comparison.

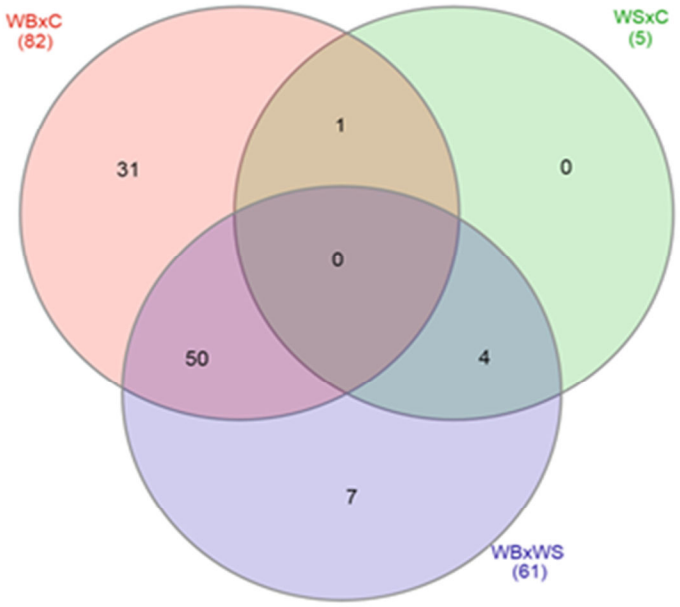


Figure 7 *- Venn diagram showing the number of miRNAs differentially expressed in comparisons between each contrast.*

3.3.4 Functional annotation

Once the DE miRNAs were identified, the sRNAtoolbox and ShinyGO tools were used to predict the target genes for these miRNAs.

3.3.5 White Striping-affected versus control group

Evaluating the five miRNAs DE in this comparison (Table 1), 2176 target genes were found in the chicken genome, out of which 2131 were previously annotated and used for gene ontology analysis. Eight pathways were enriched with the target genes predicted for WS (Table 4, Supplementary File 1 – Table S4), with autophagy and endocytosis as the most significant pathways.

Table 4 *- Metabolic pathways regulated by target genes of differentially expressed miRNAs in 28-day-old broilers affected by White Striping compared to the control group.*

Pathway	Identified Genes	Pathway Genes	FDR
Autophagy	33	126	7.8E-04
Endocytosis	49	225	7.8E-04
Insulin signaling pathway	29	114	1.7E-03
FoxO signaling pathway	28	119	7.6E-03
ErbB signaling pathway	20	76	1.0E-02
Cell cycle	25	114	3.3E-02
MTOR signaling pathway	28	137	4.2E-02
Metabolic pathways	185	1304	4.2E-02

3.3.6 Wooden breast-affected versus control group

Out of 82 DE miRNAs obtained from this contrast, 7148 target genes were found in the chicken genome, which enriched 20 metabolic pathways, such as Ubiquitin mediated proteolysis, Protein processing in endoplasmic reticulum, Cell cycle, Endocytosis, Autophagy, Insulin signaling pathway and *FoxO* signaling pathway (**Table 5**, **Supplementary File 1 – Table S5**).

Table 5 *- Metabolic pathways regulated by target genes of miRNAs differentially expressed in Wooden Breast compared to the control group*

Pathway	Identified Genes	Pathway Genes	FDR
Metabolic pathways	644	1304	7.8E-15
Endocytosis	130	225	2.4E-07
Ubiquitin mediated proteolysis	80	129	2.7E-06
Protein processing in endoplasmic reticulum	89	149	4.5E-06
Peroxisome	50	79	1.9E-04
Autophagy	73	126	1.9E-04
Cell cycle	67	114	2.0E-04
Fatty acid metabolismo	35	51	2.5E-04
Cellular senescence	77	137	3.4E-04
Salmonella infection	117	226	5.0E-04
Biosynthesis of cofactors	68	120	5.8E-04
Lysosome	64	114	1.2E-03
Insulin signaling pathway	64	114	1.2E-03
Fatty acid degradation	23	32	1.3E-03
FoxO signaling pathway	66	119	1.3E-03
Tight junction	78	145	1.3E-03
Amino sugar and nucleotide sugar metabolis	29	45	3.6E-03
Biosynthesis of nucleotide sugars	24	36	5.0E-03
Cysteine and methionine metabolism	27	42	5.0E-03
Carbon metabolismo	53	96	5.0E-03

3.4 DISCUSSION

In recent years, several studies have been conducted trying to understand the molecular mechanisms involved with the appearance of chicken pectoral myopathies (Emami *et al*., 2021; Lake *et al*., 2021; Malila *et al*., 2022; Marchesi *et al*., 2018; Marciano *et al*., 2021; Velleman *et al*., 2022). in especial with wooden breast disturb (Brothers *et al*., 2019; Emami *et al*., 2021; Hosotani *et al*., 2020; Maharjan *et al*., 2021; Malila *et al*., 2021; Papah and Abasht, 2019; Shu *et al*., 2021; Wang *et al*., 2021). However, the impact of miRNAs on the onset and differentiation of both WS and WB myopathies has not been widely explored (Shu *et al*., 2021).

The regulatory role of miRNAs in myopathies has been previously reported in humans (Chen *et al*., 2009; Parkes *et al*., 2015). However, only one study has investigated their role in chickens (Shu *et al*., 2021). Our study used young broilers of 28 days of age, allowing the identification of WS and WB in initial stages, which could help to understand the mechanisms most related with the less severe conditions. It is important to emphasize that although the experiment was carried out with 28-day-old

broilers, it was difficult to obtain control animals, i.e., with breast samples showing none or slight macroscopic and histological lesions of myopathies. This fact reinforces the high incidence of WS and/or WB observed in fast-growing commercial lines of chickens, even before slaughter age. This is exemplified by the fact that only three samples out of the 30 collected were classified as controls.

In the differential expression analysis, five miRNAS were DE between WS-affected and control groups, and 82 were DE between WB-affected and control group. Some of them, such as gga-miR-146-5p, gga-miR-29, gga-miR-21-5p, gga-miR-133a-3p and gga-miR-133b have already been associated with WB in broilers at 42 days of age (Shu *et al*., 2021), which were also DE in our study in WB-affected broilers at 28 days of age. For the first time, miRNAs were associated with the regulation and differentiation of WS myopathy in broilers. The upregulation of miR-429-3p had already been correlated with *LPIN1* downregulation, promoting abdominal fat accumulation via the *PPARγ* Pathway (Chao *et al*., 2020). Furthermore, four DE miRNAs in our study (gga-miR-375, gga-miR-200a-3p, gga-miR-200b-3p and gga-miR-429-3p) in the WS-affected compared with the control group, were also DE when Chao *et al*. (2020) evaluated high-fat and low-fat chickens. Hence, we have found miRNAs associated to the regulation of adipogenesis, a key biological process for the development of the WS phenotype.

Once the DE miRNAs were identified, the target genes were predicted, and metabolic pathways were submitted to functional analysis. In this way, the discussion was firstly structured in the common pathways involved with WB and WS, to elucidate the shared mechanisms between the two conditions. Secondly, the exclusive pathways in each myopathy were explored. Out of the six common pathways, five of them were chosen to be discussed: autophagy, insulin signaling pathway, FoxO signaling, cell cycle and endocytosis. Considering those exclusive in each myopathy, two were enriched only in the WS-affected compared with the control group: the ERBB and *mTOR* signaling pathways (**Table 4**). For the WB-affected compared to the control group, 14 pathways were enriched, of which two of them (with lower FDR values; Table 5) will be discussed: ubiquitin mediated proteolysis and protein processing in endoplasmic reticulum.

3.4.1 Shared metabolic pathways enriched among WB and WS-affected compared with the control group

3.4.1.1 Autopaghy

This was one of the most significant metabolic pathways involved in both conditions compared with the control group (**Tables 4 and 5**). Autophagy is a type of vesicular degradation associated with lysosomes (Nakatogawa, 2020), which can be activated in response to cell stress (Levine and Kroemer, 2008; Mizushima and Klionsky, 2007; Russell *et al*., 2013). Once this mechanism is deregulated, its upregulation may degrade healthy tissues, causing degenerative diseases (Klionsky *et al*., 2021). When downregulated, it can generate an accumulation of harmful substances to the body allowing the replication of defective cells (Klionsky *et al*., 2021).

Among the DE miRNAs for the WB vs control contrast, gga-miR-155, gga-miR-146b and gga-miR-222 can be highlighted. These miRNAs have already been identified as upregulated in broilers affected with WB at 42 days of age (Shu *et al*., 2021). Furthermore, it has also been reported that those miRNAs, when highly expressed, may be involved in at least 10 muscle disorders in humans (Eisenberg *et al*., 2007). These findings evince the role of these miRNAs with WB development. In our study, gga-miR-155 and gga-miR-146b were upregulated in WB when compared to the control group. miR-155 is responsible for suppressing biogenesis and affecting the expression of myocyte-specific enhancer factor 2A (*MEF2A*) and myocyte-specific enhancer factor 2C genes (*MEF2C*) (Singh *et al*., 2020). It also promotes inflammation and autophagy, and its inhibition was associated with decreased levels of autophagy in cells (H. Chen *et al*., 2019; Wan *et al*., 2019). miR-146 regulates myoblast differentiation by acting on the Mothers Against Decapentaplegic Homolog 4 (*SMAD4*), Neurogenic Locus Notch Homolog Protein 1 (*NOTCH1*) and High Mobility Group Protein *HMGI-C* (*HMGA2*) genes (Singh *et al*., 2020). In the current study, miRNAs 222b-3p and gga-miR-222b-5p were upregulated only in WB-affected compared to control group and their functions have been related to the regulation of apoptosis (Byrd and Brewer, 2013), a biological process that has already been associated with WS development (Marchesi *et al*., 2018).

According to the ShinyGO tool, the autophagy pathway is regulated by 126 genes, and 73 target genes were enriched from the WB vs control comparison, while

33 were enriched from the WS vs control contrast. A target gene predicted exclusively for the WB-affected group was the autophagy related 12 gene (*ATG12*), which encodes a protein involved in formation of the autophagic vesicles formation (Geng and Klionsky, 2008). Additionally, other exclusive targets for WB were *CASP8* and FADD Like Apoptosis Regulator (*CFLAR*). *CFLAR* is an apoptosis regulator, and its actions are related to the coordination of autophagy, apoptosis, and necroptosis processes (He and He, 2013). Since the *CFLAR* mRNA could be degraded by the activity of miRNAs, it is possible to observe an increase in tissue autophagy. Specifically, the gga-miR-15b-3p, a miRNA that was DE only in the WB vs control contrast, has a target in the Autophagy related 5 (*ATG5*) gene, which is related to mitochondrial quality control after oxidative damage and structural maintenance in autophagosomes (Ye *et al*., 2018). It is possible that gga-miR-15b-3p and other miRNAs may be influencing the autophagy process in pectoral myopathies-affected broilers. It has already been described that WB-affected chickens had a significant increase in apoptosis rates in hepatocytes compared to unaffected animals (Xing *et al*., 2021). The autophagic profile may contribute to the development of liver and neurodegenerative diseases, as well as myopathies (Mizushima and Klionsky, 2007), reinforcing the idea that the dysregulation of this pathway could predispose broilers to the myopathies' occurence.

There are several degenerative diseases related to failures in the autophagy mechanism (Deneubourg *et al*., 2022; Pagano *et al*., 2015). In WS and WB disorders, the presence of degenerative lesions is observed in the evaluated tissues, such as areas of necrosis and degeneration, which indicates the occurrence of muscle damage with an endogenous origin, and that could be explained by autophagy related processes (Prisco *et al*., 2021; Sihvo *et al*., 2014). Furthermore, the inhibition of genes related to autophagy and the appearance of cardiomyopathies is already known (Henning and Brundel, 2017). Failures in the autophagy process was also related to fibrotic disorders (Racanelli *et al*., 2018). Moreover, the miR-29c and miR-223 were previously associated with myopathies development and fibrotic disturbs in humans (Deng *et al*., 2017; Parkes *et al*., 2015). In our study, these two miRNAs were upregulated in the WB compared with the control group. Both myopathies, WS and WB were characterized by fibrotic tissue and other types of degenerative lesions (Kuttappan *et al*., 2013; Trocino *et al*., 2015), which can indicate that the regulation of the autophagic pathway is important for the two breast myopathies predisposition.

3.4.1.2 Insulin signaling pathway

This pathway acts on glucose homeostasis, which is important for animal growth (McMurtry *et al*., 1997). In addition to regulating glucose balance, insulin receptors control several biological functions, receiving signals to activate glycogen and protein synthesis (Carvalheira *et al*., 2002), acting on the liver metabolism, mantaining adipose tissue and mediating growth factors activity through insulin-like growth factor 1 and 2 (*IGF-1* and *IGF-2*) (Ebrahimi *et al*., 2019). Moreover, WB and WS-affected chickens have an increased lipid content in the pectoral muscle (de Almeida Assunção *et al*., 2020; Kuttappan *et al*., 2013; Xing *et al*., 2021), which can indicate that the augmentation of fat deposition could be related with the insulin pathway regulation. Ebrahimi *et al*. (2019) found that post-transcriptional expression mechanisms act as regulators of the insulin pathway resulting in the manifestation of disorders such as insulin resistance and obesity.

In our study, 64 genes in the insulin signaling pathway were predicted as target from DE miRNAs between WB and control groups, and 29 between WS vs control contrast. Of these, 28 genes were common for both contrasts, while WB-affected group had 36 exclusive targets and the WS-affected group had only one exclusive target gene. In the WB vs control comparison, two members of the suppressor of cytokine signaling (*SOCS*) family, *SOCS3* and *SOCS4* were enriched. *SOCS* proteins regulate the transduction of cytokine signals by binding to their receptors or by directing proteins for degradation. *SOCS3* acts more on inflammatory processes, while *SOCS4* is involved in the regulation of hormones, such as insulin and growth factors (Sobah *et al*., 2021). Studies have shown that the increased expression of miR-203 decreases *SOCS3* levels in humans, evincing the translational control by miRNAs over *SOCS* gene members (Lin *et al*., 2019). The gga-miR-203 was DE between WB and control groups, being upregulated in the affected group. This miRNA is also known as one of the key regulators of insulin sensitivity and glucose tolerance, and it is involved in the subcutaneous white adipose tissue accumulation (Guo *et al*., 2019). Our findings reinforce the hypothesis that an impaired glucolipotoxicity is involved in the etiology of wooden breast and other breast myopathies in broilers (Lake and Abasht, 2020).

Another exclusive target for WB was the *CBL Proto-Oncogene B* (*CBLB*) gene, which acts in the proteasome-mediated protein degradation (Tang *et al*., 2019). The *CBLB* gene is the target of miR-29 degradation (C. Li *et al*., 2018).In our study, several miRNAs of this family were upregulated in the WB-affected group, including gga-miR-29a-5p, gga-miR-29a-3p, gga-miR-29c-3p, gga-miR-29b-1-5p and gga-miR-29b-3p. They were previously found to be DE in WB-affected broilers from a commercial line at 42 days of age, and it is believed that the gga-miR-29 has a role in the WB development through energy metabolism regulation (Shu *et al*., 2021).

Insulin resistance is a well-established factor in metabolic disorders (Ebrahimi *et al*., 2019), and it has been shown that miRNAs can regulate the expression of the insulin pathway and insulin resistance (Chakraborty *et al*., 2014). WB-affected chickens have liver damage, such as lipid accumulation, intrahepatic hemorrhages, inflammation, and abnormal enzyme activities (Xing *et al*., 2021). It has been suggested that lipidosis may be related to problems in insulin signaling in the liver (Ebrahimi *et al*., 2019), which was also highlighted by Lake and Abasht (2020). Therefore, our study pointed out that genes in the insulin pathway could be regulated by miRNAs and may contribute to the occurrence of WS and WB in broilers. The miR-15b has already been directly associated with insulin resistance (Yang *et al*., 2015) and here, we found that gga-miR-15b-3p was among the DE miRNAs between WB and control group. Furthermore, the gga-miR-222b-3p and gga-miR-222b-5p were upregulated in the WB group, and its upregulation has already been associated with induced insulin resistance in mice (Ono *et al*., 2018). These findings indicate that these mechanisms possibly alter the insulin pathway also in broiler chickens, facilitating the myopathies occurrence.

3.4.1.3 FoxO signaling pathway

This signaling pathway is a family of transcriptional factors family responsible for controlling cell differentiation, proliferation, and survival (Potente *et al*., 2005). FoxO members are deeply involved with insulin receptors (Puig and Tjian, 2006) and gluconeogenesis (Wolfrum *et al*., 2003). It has been shown that there is an increase in lipid content in PMM of WB and WS-affected broilers (de Almeida Assunção *et al*., 2020; Kuttappan *et al*., 2013; Trocino *et al*., 2015). A total of 28 and 66 target genes

from the WS vs control and WB vs control, repectively, were enriched in the *FoxO* signaling pathway by the ShinyGO tool.

Several miRNAs are regulators of the *FoxO* pathway, including the miR-146b, which suppresses the *FoxO1* and *FoxO3* genes, and promotes adipogenesis in the tissues (Ahn *et al*., 2013). An experiment with miR-146b mouse knocked down resulted in a significant reduction in body weight and fat (Ahn *et al*., 2013). In the current study, gga-miR-146b-5p and gga-miR-146b-3p were overexpressed in the WB-affected group, which could be related with the increase in body weight and adipose tissue. On the other hand, the miR-130 suppresses adipogenesis (Kajimoto *et al*., 2006) and, in our study, gga-miR-130a-3p was downregulated in WB-affected broilers, which could favor the lipid deposition.

FoxO1 and *FoxO3* genes are also related with vascular development (Potente *et al*., 2005), and when they are not expressed, animals develop severe anomalies in the cardiovascular system (Hosaka *et al*., 2004). The impairment of vascular tissues has already been associated with myopathic conditions (Boerboom *et al*., 2018). FoxO signaling is also activated in response to stress and *FoxO3* is involved with the induction of autophagy(Ronnebaum and Patterson, 2010). Studies have shown that miR-132 regulates *FoxO3* expression, acting as anti-hypertrophic and pro-autophagic (Ucar *et al*., 2012). We highlight that gga-miR-132a-5p, gga-miR-132c-5p and gga-miR-132c-3p were upregulated in the WB-affected group when compared to the control group. miR-30d also targets the *FoxO3* gene and is involved with reduced inflammatory cell death in several tissues (Li *et al*., 2014). In our study, gga-miR-30d, as well as other members of this family, such as gga-miR-30a-3p, gga-miR-30e-5p, gga-miR-30a-5p, gga-miR-30c-5p, and gga-miR-30c-1-3p were downregulated in WB-affected group.

3.4.1.4 Cell cycle

In the ShinyGO tool, 114 genes are involved with the cell cycle pathway. Out of those, 25 and 67 were targets for DE miRNAs when the WS and WB-affected groups were compared with the control group. Twenty-three target genes were shared between the two comparisons. Cells respond to structural damage by interrupting cell cycle and recruiting repair proteins, and when repairment is not possible, cell death mechanisms are activated (Finn *et al*., 2012). Several miRNAs

are associated with cell cycle, such as miR-15 (Bonci *et al*., 2008) and let-7 family members (Johnson *et al*., 2007). In our study, gga-miR-15b-3p was upregulated and gga-let-7b was downregulated in WB-affected broilers. Furthermore, some miRNAs are related to cell cycle progression (Bueno and Malumbres, 2011), and act in myogenic differentiation, such as those of the miR-133 family (Chen *et al*., 2009; Kato *et al*., 2009). Several miRNAs were downregulated in WB-affected broilers, such as gga-miR-133c-5p, gga-miR-133a-5p, gga-miR-133a-3p, gga-miR-133c-3p and gga-miR-133b. The expression profile of these miRNAs may affect muscle development in chickens, which may be associated with the development of myopathic condition.

The miR-223, also DE between WB and control group, includes target genes from the Origin Recognition Complex Subunit family (*ORC1*, *ORC2*, *ORC3*, *ORC5* and *ORC6*) ("TargetScanHuman 8.0," n.d.). Those genes act in DNA replication and tend to have stable expression throughout the cell cycle (Duncker *et al*., 2009). However, in our study, only *ORC5* was enriched in the functional analysis. The miRNAs play a crucial role in the control of cell cycle regulatory genes, and changes in the miRNA's expression profile have been associated with the onset of several diseases (Bueno and Malumbres, 2011). Cell cycle is also driven by cellular stress, including hypoxia and DNA damage (Cui *et al*., 2021), pathways that have previously been associated with the occurrence of breast myopathies in chickens (Marchesi *et al*., 2018).

3.4.1.5 Endocytosis

The endocytosis pathway is controlled by 225 genes, according to the ShinyGO tool. A total of 49 and 113 target genes predicted by miRNAs DE in the WS and WB-affected compared with the control group, respectively, enriched in this pathway. Out of those, 47 genes were shared by the two comparisons. The gga-miR-142-3p has already been described for acting in the endocytosis (Martin-Alonso *et al*., 2018), and regulating actin in the cytoskeleton (Chapnik *et al*., 2014). Our results identified this miRNA upregulated in chickens affected with WB.

The Epidermal Growth Factor Receptor Pathway Substrate 15 (*EPS15*) gene was predicted to be a target exclusively for DE miRNAs when WB-affected group was compared with the control group. The regulation of this gene is very important for

muscle development due to its activity in mitosis, and the suppression of this gene is associated with endocytosis inhibition (Salcini *et al*., 1999). The altered endocytosis has already been associated with the development of congenital myopathy and defects in the skeletal muscle of mice (Durieux *et al*., 2010), corroborating our study that pointed out that this pathway is significant for myopathic conditions.

One of the most popular endocytosis mechanisms is phagocytosis (Grant and Donaldson, 2009). Broilers affected with myopathies have increased phagocytosis in muscle fibers (Kuttappan *et al*., 2013), indicating a possible change in the endocytic pathway. This pathway stands out in cell adhesion (Paterson *et al*., 2003). Imbalances in endocytosis may result in less adhesion between cells. This type of change was observed in the histopathological analyses of the pectoral muscle of chickens affected with WB and WS (loose arrangement of muscle fibers), suggesting a failure in the mechanism of cell adhesion in these animals (Sihvo *et al*., 2014).

Endocytic recycling is very important for the fusion of myoblasts that occurs during muscle growth, regeneration, and repair (Erickson *et al*., 1997). These mechanisms also control plasmatic membrane proteins in cell division, cytoskeletal reorganization, cell adhesion and other important activities (Pajcini *et al*., 2008). Endocytosis also affects *ERBB* receptors, altering the cell growth (Yarden and Sliwkowski, 2001). The *ERBB* pathway was also significant for the WS-affected broilers and could co-regulate both myopathies.

3.4.2 Exclusively enriched pathways in the WS-affected compared with the control group

3.4.2.1 ERBB signaling pathway

The *ERBB* family, within other functions, guides cell-cell interactions in tissues and organ formation during animal growth (Burden and Yarden, 1997). Most cells have more than one type of *ERBB* receptors (Tzahar *et al*., 1996). This signaling pahtway differs from other growth factor pathways due to its anchoring potential for signaling in several proceses, such as motility, adhesion, and cell death (Yarden and Sliwkowski, 2001). About 20 target genes were enriched to this pathway according to the DE miRNAs between WS-affected and control group. Some of them were from

MAPK family: *MAPK10*, *MAP2K4* and *MAPK9*. This gene family acts in proliferation, differentiation, transcription regulation and cell development (Cargnello and Roux, 2011). In the current study, miR-375 was downregulated in the WS-affected group, and its target genes *ERBB2* and *MAPK* were involved in fat metabolism and considered as markers for adipocytes (Xie *et al*., 2022). *ERBB* signaling becomes pathological when its activation is out of balance. Parker *et al*. (2020)(Parker *et al*., 2020) reported that high levels of *ERBB* proteins are related to kidney disease, and the overstimulation of these genes lead to an epithelial hyperproliferation, followed by inflammation and fibrosis. The fibrosis is one of the hallmarks of WS.

The *ERBB* signaling pathway might be connected with changes developed in WS by the identification of DE miRNAs that are characterized by suppressing the expression of *ERBB* receptors (L. Li *et al*., 2018). mir-375 has a role in trigger apoptosis through *ERBB2* receptor expression and its downregulation triggers cell proliferation and tumorigenesis (L. Li *et al*., 2018). The downregulation of this miRNA could favour the cell proliferation in the WS-affected broilers. Furthermore, an aberrant expression of *ERBB* pathway were also related with the inflammation and fibrosis appearance, two characteristics presented in chickens affected with WS (Kuttappan *et al*., 2013; Trocino *et al*., 2015).

3.4.2.2 mTOR signaling pathway

The *mTOR* signaling pathway is key in biological processes related to cell growth, survival, aging and healthy muscle development (Léger *et al*., 2006; Wullschleger *et al*., 2006). The *mTOR* positive regulation is related with muscular hypertrophy (Bodine *et al*., 2001; Léger *et al*., 2006). The *mTOR* integrates information from the extracellular environment, such as availability of nutrients and energy, into intracellular stimuli promoting protein synthesis (Hay and Sonenberg, 2004). This pathway also responds to cellular stress by disrupting protein production, and its activation increases intensely with the presence of insulin and growth factors, such as *IGF-1* (Avruch *et al*., 2006). The *mTOR* is also involved in the regulation of insulin sensitivity (Hodson *et al*., 2017). Twenty-eight target genes from the DE miRNAS between WS and control group enriched in the *mTOR* sinaling pathway. miRNAs regulate gene expression of various components of this pathway. The miR-375 plays an important role in the *mTOR* pathway suppressing cell proliferation and

apoptosis (Sun *et al*., 2019), and also inhibiting cellular signals of osteogenesis and adipogenesis (L. R. Chen *et al*., 2019). In our study, miR-375 was upregulated in the control group, so it can inhibit adiopogenesis in normal broilers and allowing a bigger lipid deposition in muscles from WS-affected broilers. This miRNA was also related with an increase in the level of *mTOR*-mediated autophagy (Yuan *et al*., 2018), controlling adipogenesis. In our study, miR-375 was downregulated in WS-affected broilers, as well as two members of the miR-200 family. Therefore, the lower expression of these miRNAs in the WS-affected broilers might be contributing with the increased adipogenesis in those PMMs.

Another miRNA DE in the WSxC contrast was the gga-miR-429-3p, which was downregulated in the WS-affected broilers. Members of this family are downregulated during hypoxia (Janaszak-Jasiecka *et al*., 2016), which may suggest an association with increased levels of hypoxia in chickens with WS (Kuttappan *et al*., 2013). Among all the functions that have been identified for the *mTOR* pathway, it also regulates glucose resistance, cell proliferation and autophagy. Therefore, our results reinforce the hypotheses that WS might also be caused by a disruption of the gluco and lipid metabolism, which corroborate with the Lake and Abasht (2020)(Lake and Abasht, 2020) hypothesis.

3.4.3 Exclusively enriched pathways in the WB-affected compared with the control group

3.4.3.1 Ubiquitin-mediated proteolysis (UP)

Eukaryotic cells have a highly specific signaling system for intracellular proteins that need to be degraded (Pickart, 2001) to ensure the quality of biological processes in the cell (Koepp, 2014). The Ubiquitin-Proteasome (UP) system degrades intracellular proteins and structures dispersed in the cytosol with high specificity (Luzio *et al*., 2007). The UP pathway contains 129 genes, 80 of which were predicted to be targets of regulation by the miRNAs DE between WB and control groups. miRNA regulation of ubiquitin-mediated proteolysis factors plays an important role in adaptive energy metabolism (Nie *et al*., 2022). The miR-122 which was identified in hypoxic skeletal muscles, participates in the Ubiquitin-mediated proteolysis pathway, and contributes to the manifestation of musculoskeletal

diseases, such as myofibrillar degradation (Yan *et al*., 2022). Here, we identified two downregulated members of the miR-122 family in the WB-affected broilers, the gga-miR-122-5p and the gga-miR-122b-5p. Possibly, both miRNAs are linked to the histological alterations, such as high levels of necrosis and disorganization of muscle fibers observed in the pectoral muscle of WB-affected animals.

Abnormal activity of the UP pathway can induce pathological conditions in organisms, such as muscle atrophy (Khalil, 2018) accumulation of oxidized proteins (Ferrington *et al*., 2005). It can also trigger several anomalies in the skeletal muscle, such as degenerative and regenerative alterations, basophilic infiltrations, vacuolated fibers, disorganized myofibrils and increased interstitial space (Kitajima *et al*., 2014). Several of these tissue alterations are reported in WB myopathy (Sihvo *et al*., 2014). Thus, it is reasonable to assume that miRNAs influence the regulation of the proteolysis pathway in WB-affected muscle.

The UP system is very active during myogenesis, and it is responsible for several characteristics acquired in muscular development (Gardrat *et al*., 1997). The proteolytic pathway controls proteins involved in diverse cellular functions, including cell cycle (D'Angiolella *et al*., 2013) and myoblast differentiation (Gardrat *et al*., 1997), thereby suggesting a potential association with the onset of myopathic tissue disorder.

3.4.3.2 Processing of proteins in the endoplasmic reticulum

The endoplasmic reticulum (ER) is responsible for the production of integral and secretory proteins that constitute the plasma membrane (Jan *et al*., 2014). This production is carried out by ribosomes in the ER membrane. After synthesis, such proteins undergo conformational modifications with the assistance of chaperones and folding enzymes(Braakman and Hebert, 2013). Proteins that are not correctly folded are detected by the cell and sent to ER-Associated Protein Degradation (ERAD) (Ruggiano *et al*., 2014). When ERAD is not efficient, the cell can accumulate dangerous proteins compromising its functions, and this condition was already associated with several diseases (Walter and Ron, 2011). On the other hand, with excessive ERAD activity, the cell may be harmed by the degradation of important proteins (Ruggiano *et al*., 2014).

The protein processing pathway in the ER comprises 149 genes. In our study we found 89 of them as targets of miRNAs DE between WB and the control group. Studies indicate that there are miRNAs that inhibit the translation of mRNAs in the ER, directly interfering in the synthesis and processing of proteins and influencing the development of organisms (Li *et al*., 2013). miRNAs form a complex regulatory network on this pathway, for instance, miR-122 can act in UP and apoptosis (Byrd and Brewer, 2013). The gga-miR-122-5p was downregulated in broilers with WB in our study, indicating a possible influence of this miRNA on the manifestation of WB.

Some miRNAs respond to ER stress conditions by regulating pro-apoptotic genes and influencing cell death (Byrd and Brewer, 2013), including miR-29 (Wang and Lee, 2009). In our study, we found six mirRNAs of this family overexpressed in broilers with WB compared to the control group (gga-miR-29a-5p, gga-miR-29a-3p, gga-miR-29c-3p, gga-miR-29b-1-5p and gga-miR-29b-3p). Furthermore, the gga-miR-455-5p, a miRNA linked to transcription factors involved in ER homeostasis (Byrd and Brewer, 2013), was also overexpressed in broilers with WB. Although this miRNA has not previoulsly been associated with myopathic disorders, it is possible that it affects the ER homeostasis in WB-affected chickens.

The protein processing in the ER is linked with the UP system, which marks contents that must be degraded by the ERAD (Carvalho *et al*., 2006). ER stress is associated with degenerative disorders and myopathies, and may originate from glucose and nutrient deprivation, hypoxia, inflammation, and oxidative stress. High levels of ER stress have already been related to the development of myopathies (Li *et al*., 2009; Vitadello *et al*., 2010). Myopathic features, such as cell death, regenerative changes and muscle weakness were related to stress in the ER (Fréret *et al*., 2013). ER stress causes errors in protein folding, activating UP in response to degrade these misfolded proteins in processes related to autophagy, necrosis, and apoptosis, in addition to triggering inflammatory responses in the tissue (Hotamisligil, 2010; Rayavarapu *et al*., 2012).

Several changes that cause ER stress are observed in chickens with WB, such as hypoxia and oxidative stress (Sihvo *et al*., 2014; Xing *et al*., 2021). These changes can act as a source of ER stress in the pectoralis muscle of these animals, leading to disruptions in protein synthesis and processing in the ER. All these evidences support the notion that the ER protein processing pathway plays a key role in the manifestation of WB.

From all those DE miRNAs, it was possible to observe several metabolic pathways involved with pectoral myopathies in broiler chickens. Some of them, such as insulin signaling, *mTOR* signaling and pathways related to lipid regulation reinforce findings from previous studies (Marchesi *et al*., 2018; Mutryn *et al*., 2015), while some other pathways, such as proteolysis mediated by ubiquitin, tigh junction and cell senescence, are being related here for the first time.

In our study, we found some types of injuries, with different frequency and intensities in both WS and WB myopathies. The miRNAs expression profile reveals only one miRNA (gga-miR-1769-5p) DE simultaneously for the two conditions compared to the control, demonstrating that these molecular mechanisms may be underlying some of the differences between these two myopathies. Our results also provide a novel evidence that miRNAs are involved in the regulation of WS and in the differentiation of both WS and WB myopathies. Furthermore, the WB-affected broilers presented a high number of DE miRNAs in comparison with the control group, strongly suggesting that animals with WB may be more influenced by miRNAs control than broilers with WS. Therefore, our results evince that both myopathies are somehow regulated by an epigenetic factor. Moreover, functional enrichment and ontology analysis of DE miRNA target genes pointed to some metabolic pathways that are involved in the manifestations of those myopathies.

3.5 CONCLUSIONS

In our study, we successfully identified miRNAs differentially expressed between normal broilers and thouse affected with breast myopathies at 28 days of age. Eighty-two miRNAs were DE between WB-affected and control group, while only five were DE between WS-affected and control groups in this age. Additionally, we have also predicted 17 new miRNAs expressed in the broilers pectoral muscle, which can be investigated in future studies. Furthermore, by analyzing the DE miRNAs, we were able to predict target genes and, consequently, the main pathways involved with both breast myopathies, such as autophagy, insulin signaling pathway, FoxO signaling pathway, cell cycle and endocytosis. Nevertheless, differentiating WB and WS, 14 pathways were involved exclusively with WB, with ubiquitin-mediated proteolysis and ER protein processing as the two most significant, while only two pathways were exclusive for WS: *ERBB* and *mTOR* signaling. These findings

highlight the miRNAs' role in energy metabolism, insulin metabolism, hypoxia, autofagy, inflammation, protein synthesis and cell proliferation mechanisms, which have already been described as important for WS and WB occurrence. Five miRNAs, gga-miR-375, gga-miR-200b-3p, gga-miR-429-3p, gga-miR-1769-5p, gga-miR-200a-3p, were involved with the WS occurrence and there were no previous studies associating miRNA expression with this condition. Furthermore, several known miRNAs were associated with the development of WB, such as gga-miR-155, gga-miR-146b, gga-miR-222, gga-miR-146-5p, gga-miR- 29, gga-miR-21-5p, gga-miR-133a-3p and gga-miR-133b, while most of them had not yet been associated with the development of this myopathy in broilers. Overall, our study provides insights into the miRNAs and pathways involved with the occurrence of WS and WB myopathies in 28-day-old broilers.

3.6 METHODS

3.6.1 Animals and Sample collection

This work was carried out at the Embrapa Swine and Poultry National Research Center, located in Concórdia - Santa Catarina State, Brazil. Thirty Ross male broilers were reared in boxes and managed according to the commercial line recommendations, receiving standard feed and water ad libitum. The broilers were euthanized by cervical dislocation at 28 days of age, following the practices recommended by the Committee on Ethics in the Use of Animals (CEUA protocol 08/2019). Immediately after slaughter, the pectoralis major muscle (PMM) of the chickens were visually evaluated for the presence or absence of WS and WB, according to KUTTAPPAN *et al*. (2013) and SIHVO; IMMONEN; PUOLANNE (2014). Approximately 1 cm2 of the PMM was collected from the cranial region for histopathological and miRNA sequencing analyses.

3.6.2 Histopathological analyses

For the histopathological analyses, the collected samples were fixed in 4% paraformaldehyde until processing. Tissues were cut into 5 mm sections, dehydrated in alcohol, diaphanized and embedded in paraffin. Then, tissues were cut into 3 µm

sections, mounted in slides and stained with hematoxylin and eosin for morphologic evaluation and identification of myopathic lesions.

3.6.3 RNA extraction, library preparation and sequencing

RNA extraction was performed from 100 mg of pectoral muscle samples, which were ground with a mortar and pestle in liquid nitrogen. Then, the total RNA was extracted using the Trizol protocol, according to the manufacturer's instructions. Total RNA was quantified in a BioDrop spectrophotometer (Biodrop, UK), and was considered of good quality when the OD260: OD280 ratio was greater than 1.8. The integrity of the samples was confirmed by electrophoresis for 90 minutes in a 1% agarose gel and also using a Bioanalyzer Agilent 2100 equipment, where samples with RNA Integrity Number (RIN) greater than 8 were used for downstream analyses.

The miRNA libraries were constructed using QIAseq miRNA Library kit (Qiagen, Germany) with the standard protocol. Libraries were quantified and verified in the Bioanalyzer Agilent 2100 equipment and quantitative PCR (qPCR). Sequencing was carried out in NextSeq 2000 equipment (Illumina), at the Life Sciences Core Facility (LaCTAD) of the University of Campinas (UNICAMP), in Campinas, São Paulo State, following a single-end protocol (1x75 bp).

3.6.4 Sequencing quality control and mapping

The FASTQ files were submitted to quality control (QC) analysis using the Trimmomatic tool (Bolger *et al*., 2014) in order to remove sequences with low average Phred quality score (PHRED < 20), short reads (length < 18 nucleotides) and sequences with undefined bases (identified as N). Following, the unique molecular identifiers (UMIs) were extracted and deduplicated using the UMI-tools (Smith *et al*., 2017). Then, an initial mapping using bowtie (Langmead *et al*., 2009) was performed against the Rfam database release 14 (https://rfam.org/) (Kalvari *et al*., 2018) to remove tRNA and rRNAs sequences. After that, the miRDeep2 software(Friedländer *et al*., 2012) was used to map the remaining sequences against the chicken genome (GRCg6a, accession GCF_000002315.5) to identify and quantify miRNA sequences present in the analyzed samples. Furthermore, the

miRDeep2 was also applied to discover potencial novel chicken miRNAs. For quantification of known miRNAs, FASTA files from miRBase release 22.1(Griffiths-Jones *et al*., 2006) and MirGeneDB release 2.1(Fromm *et al*., 2022, 2020) databases were used. These analyses were run in the BAQCOM automated pipeline (https://github.com/hanielcedraz/BAQCOM).

3.6.5 Reads counting, filtering, miRNA differential expression and functional annotation

The miRNA counts were obtained using the miRDeep2 software(Friedländer *et al*., 2012) and the counts were filtered using the "filterbyexpr" function from the edgeR package(Robinson *et al*., 2010) from R language (R Core Team, 2015). Then, the remaining miRNAs were also analyzed with edgeR for differential expression among the three groups (control, WS and WB). After obtaining DE miRNAs, the target mRNAs were searched using the sRNAtoolbox (Rueda *et al*., 2015) online tool, with the default parameters for the Pita, miRanda, TargetSpy and Simple Seed Analysis tools. The miRNAs target genes were submitted to gene ontology analysis with the ShinyGO software(Ge *et al*., 2020) (GE; JUNG; YAO, 2020).

3.6.6 Supplementary files

Supplementary file 1. Table S1. Total number of reads, read number after quality control (QC) and mapped reads in each evaluated sample. Table S2. miRNAs differentially expressed between Wooden Breast-affected and normal broilers (control). Table S3. miRNAs differentially expressed between Wooden Breast and White Striping-affected broilers. Table S4. Enrichment analysis using ShinyGO of target genes brom the DE miRNAs between White Striping versus Control group. Table S5. Enrichment analysis using ShinyGO of target genes brom the DE miRNAs between Wooden Breast versus Control group.

Declarations

Ethics approval and consent to participate

All animal procedures were performed in accordance with the Ethics Committee on Animal Utilization from the Embrapa Swine and Poultry National Research Center under protocol number 08/2019.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analyzed in this study are available from the corresponding author on reasonable request. The miRNA sequences are available in the SRA database under the BioProject number PRJNA950417.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by Project Number 10.19.03.034.00.00 from the Brazilian Agricultural Research Corporation (EMBRAPA). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions

AMGI, JOP, FCT and MCL conceived and designed the experiment. AMGI, JOP, MCL, FCT, LTF and MAZM were responsible for the data collection. LTF and MAZM were responsible for the histopathological analysis. AMGI, FGC, HCO, MSPD and MEC performed the miRNA sequencing analysis. MSPD performed the functional analyses. AMGI, MPSD, JOP and MCL interpreted the results and evaluated the conclusions. AMGI, MSDP, JOP and MCL wrote the manuscript. JOP was responsible for the project’s funding. AMGI and MCL supervised the work. All authors reviewed, edited, and approved the final version of the manuscript.

Acknowledgements

MSDP was sponsored by a scholarship from the Coordination for the Improvement of Higher Education Personnel/The Santa Catarina State Research Foundation (CAPES/FAPESC), Brazil and FGC received a CAPES Scholarship. HCO was a post-doctoral fellow from the National Council for Scientific and Technological Development (CNPq). LTF received a visiting specialist fellowship from CNPq/Embrapa. MCL, FCT and AMGI are recipients of a productivity fellowship from CNPq. We thank the CAPES (Finance Code 001) for the free access to the journals used in the literature review.

Authors' information (optional)

1Programa de Pós-Graduação em Zootecnia, Universidade do Estado de Santa Catarina, UDESC-Oeste, Chapecó, Santa Catarina, Brazil.

2Embrapa Suínos e Aves, Concórdia, Santa Catarina, Brazil.

3Programa de Pós-Graduação em Ciências Veterinárias, Universidade Estadual do Centro Oeste, Guarapuava, Paraná, Brazil.

4Programa de Pós-Graduação em Zootecnia, Departamento de Zootecnia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

4 CONSIDERAÇÕES FINAIS

Para que o tecido muscular se desenvolva de forma saudável ele depende de manutenção fisiológica constante, que atue equilibrando os processos de síntese e degradação muscular. Alterações nas vias que controlam esse papel normalmente estão ligadas às desordens metabólicas, como é o caso das miopatias WB e WS. miRNAs e seus genes-alvo podem ser fortes candidatos para regular o início de WB e WS que são as principais miopatias que afetam a produção avícola.

Neste estudo, utilizando frangos controle e afetados com WS e WB, foram identificados um conjunto de 303 miRNAs, dos quais, 17 são novos e 286 são miRNAs já disponibilizados pelas bases de dados públicas. Além disso, foram identificados os miRNAs diferencialmente expressos nos três contrastes possíveis (WS comparado com C, WB comparado com C e WB comparado com WS) e os seus possíveis genes alvos. A partir do grande número de genes alvos, as análises *in silico* foram realizadas para identificação das principais vias metabólicas envolvidas nessas condições.

O nosso estudo revelou que os miRNAs diferencialmente expressos, para WB e WS em relação ao controle, controlam genes em 6 vias significativas em comum para WS e WB: autofagia, via de sinalização de insulina, via de sinalização *FoxO*, ciclo celular, endocitose e vias metabólicas. Pela atuação dos miRNAs sobre essas vias ter sido diferente para os animais afetados pelos distúrbios, sugere-se que eles estejam por trás de múltiplas alterações fisiológicas e teciduais encontradas nas miopatias.

Na maioria das vezes que a miopatia WB é detectada, o animal também apresenta WS, o que torna bastante desafiador entender quais os mecanismos moleculares responsáveis por diferenciar a WB da WS. Isso também explica o fato de nosso estudo ter obtido várias vias metabólicas enriquecidas de forma significativa para as duas miopatias. Relatamos uma diferença muito maior entre o perfil de miRNAs de WB em comparação com o controle do que do grupo WS, indicando que o distúrbio do peito madeira está envolvido com alterações em mais vias fisiológicas. O perfil de miRNA pode ser um fator importante para entender a diferenciação entre as duas miopatias.

De acordo com o perfil de expressão de miRNAs observado, mecanismos de silenciamento gênico pós-transcricional guiados por microRNAs podem estar

atuando no desencadeamento das miopatias peitorais *White Striping* e *Wooden Breast* em frangos de corte aos 28 dias de idade. O melhor entendimento dos fatores que influenciam essas miopatias pode levar a estratégias para reduzir a incidência dessas patologias, e com isso melhorar a qualidade da carne do peito de frango, diminuindo as perdas do setor.

5 REFERÊNCIAS

ABPA (Brasil). **Associação Brasileira de Proteína Animal**. Produção de Aves. 2021. Disponível em: https://abpa-br.org/wp-content/uploads/2021/04/ABPA_Relatorio_Anual_2021_web.pdf. Acesso em: 24/06/2022

ABPA (Brasil). **Associação Brasileira de Proteína Animal**. Produção de Aves. 2021. Disponível em: https://abpa-br.org/wp-content/uploads/2020/05/abpa_relatorio_anual_2020_portugues_web.pdf. Acesso em: 28 abr. 2021.

AFROZE D.; KUMAR, A. ER stress in skeletal muscle remodeling and myopathies. **The FEBS Journal**, v. 286, n. 2, p. 379-398, 2019.

AHN, J. *et al*. Micro RNA-146b promotes adipogenesis by suppressing the SIRT 1-*FOXO* 1 cascade. **EMBO molecular medicine**, v. 5, n. 10, p. 1602-1612, 2013.

ALNAHHAS, N. *et al.* Genetic parameters of *White Striping* in relation to body weight, carcass composition, and meat quality traits in two broiler lines divergently selected for the ultimate pH of the pectoralis major muscle. **BMC genetics**, v. 17, n. 1, p. 1-9, 2016.

ANDERS, S.; PYL, P. T.; HUBER, Wolfgang. HTSeq—a Python framework to work with high-throughput sequencing data. **Bioinformatics**, v. 31, n. 2, p. 166-169, 2015.

AVRUCH, J. *et al*. Insulin and amino-acid regulation of *mTOR* signaling and kinase activity through the Rheb GTPase. **Oncogene,** v. 25, n. 48, p. 6361-6372, 2006.

BAILEY, R. A. *et al*. The genetic basis of pectoralis major myopathies in modern broiler chicken lines**. Poultry Science**, v. 94, n. 12, p. 2870-2879, 2015.

BARMAN, B.; BHATTACHARYYA, S. N. mRNA targeting to endoplasmic reticulum precedes ago protein interaction and microRNA (miRNA)-mediated translation repression in mammalian cells. **Journal of Biological Chemistry**, v. 290, n. 41, p. 24650-24656, 2015.

BARTEL, D. P. MicroRNAs: target recognition and regulatory functions. **Cell,** v. 136, n. 2, p. 215-233, 2009.

BENJAMINI, Y.; HOCHBERG, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. **Journal of the Royal statistical society: series B (Methodological)**, v. 57, n. 1, p. 289-300, 1995.

BLOMEN, V. A.; BOONSTRA, J. Cell fate determination during G1 phase progression. **Cellular and Molecular Life Sciences**, v. 64, n. 23, p. 3084-3104, 2007.

BODINE, S. C. *et al*. Akt/*mTOR* pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. **Nature cell biology**, v. 3, n. 11, p. 1014-1019, 2001.

BOERBOOM, G. *et al.* Unraveling the cause of *White Striping* in broilers using metabolomics**. Poultry science**, v. 97, n. 11, p. 3977-3986, 2018.

BOLGER, A. M.; LOHSE, M.; USADEL, B. Trimmomatic: a flexible trimmer for Illumina sequence data. **Bioinformatics**, v. 30, n. 15, p. 2114-2120, 2014.

BONCI, D.*et al*. The miR-15a–miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities**. Nature medicine**, v. 14, n. 11, p. 1271-1277, 2008.

BORDINI, Martina *et al*. Molecular Pathways and Key Genes Associated With Breast Width and Protein Content in *White Striping* and *Wooden Breast* Chicken Pectoral Muscle. **Frontiers in Physiology**, p. 1377, 2022.

BORDINI, M. *et al*. Weighted gene co-expression network analysis identifies molecular pathways and hub genes involved in broiler *White Striping* and *Wooden Breast* myopathies. **Scientific reports**, v. 11, n. 1, p. 1-13, 2021.

BUENO, M.; MALUMBRES, M.. MicroRNAs and the cell cycle**. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease**, v. 1812, n. 5, p. 592-601, 2011.

BRAAKMAN, I.; HEBERT, D. N. Protein folding in the endoplasmic reticulum. **Cold Spring Harbor perspectives in biology**, v. 5, n. 5, p. a013201, 2013.

BRAMBILA, G. S.; BOWKER, B. C.; ZHUANG, H.. Comparison of sensory texture attributes of broiler breast fillets with different degrees of *White Striping*. **Poultry science**, v. 95, n. 10, p. 2472-2476, 2016.

BRITSCH S. The neuregulin-I/*ERBB* signaling system in development and disease. **Adv Anat Embryol Cell Biol**. v. 190, p. 1-65, 2007.

BROTHERS, B. *et al*. RNA-seq analysis reveals spatial and sex differences in pectoralis major muscle of broiler chickens contributing to difference in susceptibility to *Wooden Breast* disease. **Frontiers in physiology**, v. 10, p. 764, 2019.

BURDEN, S.; YARDEN, Y. Neuregulins and their receptors: a versatile signaling module in organogenesis and oncogenesis. **Neuron**, v. 18, n. 6, p. 847-855, 1997.

BURT, D. W. The chicken genome and the developmental biologist. **Mechanisms of development**, v. 121, n. 9, p. 1129-1135, 2004.

BYRD, A. E.; BREWER, J. W. Micro (RNA) managing endoplasmic reticulum stress. **IUBMB life**, v. 65, n. 5, p. 373-381, 2013.

CALDER, P. C. Functional roles of fatty acids and their effects on human health. **Journal of parenteral and enteral nutrition**, v. 39, p. 18S-32S, 2015.

CALIN, G.A. *et al.* Chromosomal rearrangements and microRNAs: a new cancer link with clinical implications. **The Journal of clinical investigation**, v. 117, n. 8, p. 2059-2066, 2007.

CAMACHO, C. *et al.* BLAST+: architecture and applications. **BMC bioinformatics**, v. 10, n. 1, p. 1-9, 2009.

CARGNELLO, M.; ROUX, P. P. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. **Microbiology and molecular biology reviews**, v. 75, n. 1, p. 50-83, 2011.

CARVALHEIRA, J. B. C.; ZECCHIN, H. G.; SAAD, M. J. A. Vias de sinalização da insulina. **Arquivos Brasileiros de Endocrinologia & Metabologia**, v. 46, p. 419-425, 2002.

CARVALHO, P.; GODER, V.; RAPOPORT, T. A. Distinct ubiquitin-ligase complexes define convergent pathways for the degradation of ER proteins. **Cell**, v. 126, n. 2, p. 361-373, 2006.

CHAKRABORTY, C. *et al*. Influence of miRNA in insulin signaling pathway and insulin resistance: micro-molecules with a major role in type-2 diabetes. Wiley Interdisciplinary Reviews: **RNA**, v. 5, n. 5, p. 697-712, 2014.

CHANG, L.; CHIANG, S. H.; SALTIEL, A. R. Insulin signaling and the regulation of glucose transport. **Molecular medicine**, v. 10, n. 7, p. 65-71, 2004.

CHAPNIK, E. *et al*. miR-142 orchestrates a network of actin cytoskeleton regulators during megakaryopoiesis. **Elife**, v. 3, p. e01964, 2014.

CHEN, J-F.; CALLIS, T. E..; WANG, D-Z. microRNAs and muscle disorders. **Journal of cell science**, v. 122, n. 1, p. 13-20, 2009.

CHEN, J. F. *et al*. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. **Nature genetics**, v. 38, n. 2, p. 228-233, 2006.

CHEN, L. R. *et al*. Temporal characterization of *Wooden Breast* myopathy (“woody breast”) severity and correlation with growth rate and lymphocytic phlebitis in three commercial broiler strains and a random-bred broiler strain. **Avian Pathology**, v. 48, n. 4, p. 319-328, 2019a.

CHEN, H. *et al*. MicroRNA‐155 affects oxidative damage through regulating autophagy in endothelial cells. **Oncology letters**, v. 17, n. 2, p. 2237-2243, 2019b.

CHEN, L. *et al*. Trends in the development of miRNA bioinformatics tools. **Briefings in bioinformatics**, v. 20, n. 5, p. 1836-1852, 2019c.

CHEN, N. *et al*. Bioinformatics analysis of microRNA linked to ubiquitin proteasome system in traumatic osteonecrosis of the femoral head. **Medicine**, v. 99, n. 33, 2020.

CHEN, S. I. *et al*. Promotion effects of miR-375 on the osteogenic differentiation of human adipose-derived mesenchymal stem cells. **Stem Cell Reports**, v. 8, n. 3, p. 773-786, 2017.

CHEN, Y. *et al*. MicroRNAs and their regulatory networks in Chinese Gushi chicken abdominal adipose tissue during postnatal late development**. BMC genomics**, v. 20, n. 1, p. 1-18, 2019.

CHEN, Y.; LUN, A. TL; SMYTH, G. K. From reads to genes to pathways: differential expression analysis of RNA-Seq experiments using Rsubread and the edgeR quasi-likelihood pipeline. **F1000 Research**, v. 5, 2016.

CITRI, A.; YARDEN, Y. EGF–*ERBB* signalling: towards the systems level. **Nature reviews Molecular cell biology**, v. 7, n. 7, p. 505-516, 2006.

COLLINS, A. S. *et al*. miR-19a: an effective regulator of SOCS3 and enhancer of JAK-STAT signalling. **PloS one**, v. 8, n. 7, p. e69090, 2013.

CORE, R. Team. R: a language and environment for statistical computing, v. 3, p. 2, 2015.

CRNCEC, A.; HOCHEGGER, H. Triggering mitosis. **FEBS letters**, v. 593, n. 20, p. 2868-2888, 2019.

CUI, D. *et al*. The cross talk between p53 and *mTOR* pathways in response to physiological and genotoxic stresses. **Frontiers in Cell and Developmental Biology**, p. 3320, 2021.

D'ANGIOLELLA, V.; ESENCAY, M.; PAGANO, M. A cyclin without cyclin-dependent kinases: cyclin F controls genome stability through ubiquitin-mediated proteolysis. **Trends in cell biology**, v. 23, n. 3, p. 135-140, 2013.

DE ALMEIDA ASSUNÇÃO, A. S. *et al. Wooden Breast* myopathy on broiler breast fillets affects quality and consumer preference. **Tropical Animal Health and Production**, v. 52, n. 6, p. 3555-3565, 2020.

DE JONG, I. *et al.* Scientific report updating the EFSA opinions on the welfare of broilers and broiler breeders**. EFSA Supporting Publications**, v. 9, n. 6, p. 295, 2012.

DENEUBOURG, C.*et al*. The spectrum of neurodevelopmental, neuromuscular and neurodegenerative disorders due to defective autophagy. **Autophagy**, v. 18, n. 3, p. 496-517, 2022.

DENG, Z. *et al*. MicroRNA-29: a crucial player in fibrotic disease. **Molecular diagnosis & therapy**, v. 21, n. 3, p. 285-294, 2017.

DONALDSON, J. G.; PORAT-SHLIOM, N.; COHEN, L. A. Clathrin-independent endocytosis: a unique platform for cell signaling and PM remodeling. **Cellular signalling**, v. 21, n. 1, p. 1-6, 2009.

DOSSOU, A. S.; BASU, A. The emerging roles of *mTOR*C1 in macromanaging autophagy. **Cancers**, v. 11, n. 10, p. 1422, 2019.

DUBREZ, L. Regulation of *E2F1* transcription factor by ubiquitin conjugation. **International journal of molecular sciences**, v. 18, n. 10, p. 2188, 2017.

DUNCKER, B. P.; CHESNOKOV, I. N.; MCCONKEY, B. J. The origin recognition complex protein family. **Genome biology**, v. 10, n. 3, p. 1-8, 2009.

DURIEUX, A. C. *et al*. A centronuclear myopathy-dynamin 2 mutation impairs skeletal muscle structure and function in mice. **Human molecular genetics**, v. 19, n. 24, p. 4820-4836, 2010.

EBRAHIMI, R. *et al*. The role of microRNAs in the regulation of insulin signaling pathway with respect to metabolic and mitogenic cascades: A review. **Journal of cellular biochemistry**, v. 120, n. 12, p. 19290-19309, 2019.

EICHHORN, P. J. A. *et al*. USP15 stabilizes TGF-β receptor I and promotes oncogenesis through the activation of TGF-β signaling in glioblastoma. **Nature medicine**, v. 18, n. 3, p. 429-435, 2012.

EISENBERG, I. *et al*. Distinctive patterns of microRNA expression in primary muscular disorders. **Proceedings of the National Academy of Sciences**, v. 104, n. 43, p. 17016-17021, 2007.

EMAMI, N. K. *et al*. Hypoxia further exacerbates woody breast myopathy in broilers via alteration of satellite cell fate. **Poultry Science**, v. 100, n. 7, p. 101167, 2021.

ERICKSON, M. R. S.; GALLETTA, B. J.; ABMAYR, S. M. Drosophila myoblast city encodes a conserved protein that is essential for myoblast fusion, dorsal closure, and cytoskeletal organization. **The Journal of cell biology**, v. 138, n. 3, p. 589-603, 1997.

EWING, B.; GREEN, P. Base-calling of automated sequencer traces using phred. II. Error probabilities. **Genome research**, v. 8, n. 3, p. 186-194, 1998.

EWING, Brent *et al*. Base-calling of automated sequencer traces usingPhred. I. Accuracy assessment. **Genome research**, v. 8, n. 3, p. 175-185, 1998.

FEITOSA, R. M. M. W. *et al*. MicroRNA target prediction tools for animals: Where we are at and where we are going to-A systematic review. **Computational Biology and Chemistry**, p. 107729, 2022.

FERREIRA, T. Z. *et al.* An investigation of a reported case of *White Striping* in broilers. **Journal of Applied Poultry Research**, v. 23, n. 4, p. 748-753, 2014.

FERRINGTON, D. A.; HUSOM, A. D.; THOMPSON, L. V. Altered proteasome structure, function, and oxidation in aged muscle. **The FASEB journal**, v. 19, n. 6, p. 1-24, 2005.

FINN, K.; LOWNDES, N. F.; GRENON, M. Eukaryotic DNA damage checkpoint activation in response to double-strand breaks. **Cellular and Molecular Life Sciences**, v. 69, n. 9, p. 1447-1473, 2012.

FIRE, A. *et al.* Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. **Nature**, v. 391, n. 6669, p. 806-811, 1998.

FRÉRET, M. *et al*. Overexpression of MHC class I in muscle of lymphocyte-deficient mice causes a severe myopathy with induction of the unfolded protein response. **The American Journal of Pathology**, v. 183, n. 3, p. 893-904, 2013.

FRIEDLÄNDER, M. R. *et al.* miRDeep2 accurately identifies known and hundreds of novel microRNA genes in seven animal clades. **Nucleic Acids Research**, v. 40, n. 1, p. 37-52, 2012.

FROMM, B. *et al*. MirGeneDB 2.0: the metazoan microRNA complement. **Nucleic Acids Research**, v. 48, n. D1, p. D132-D141, 2020.

FROMM, B. *et al*. MirGeneDB 2.1: toward a complete sampling of all major animal phyla. **Nucleic Acids Researc**h, v. 50, n. D1, p. D204-D210, 2022.

GAPP, K. *et al.* Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. **Nature neuroscience**, v. 17, n. 5, p. 667-669, 2014.

GARDRAT, F. *et al*. Proteasome and myogenesis. **Molecular** biology reports, v. 24, n. 1, p. 77-81, 1997.

GATENBY, R. A.; GILLIES, R. J. Why do cancers have high aerobic glycolysis?. **Nature reviews cancer**, v. 4, n. 11, p. 891-899, 2004.

GE, S. X.; JUNG, D.; YAO, R. **ShinyGO: a graphical gene-set enrichment tool for animals and plants**. Bioinformatics, v. 36, n. 8, p. 2628-2629, 2020.

GEBERT, L. F. R.; MACRAE, I. J. Regulation of microRNA function in animals. **Nature reviews Molecular cell biology**, v. 20, n. 1, p. 21-37, 2019.

GENECARDS. ***FN1* Gene *- Fibronectin 1*.** Disponível em: <https://www.genecards.org/cgi-bin/carddisp.pl?gene=FN1>. Acesso em: 07 de dez. 2022.

GENG, J.; KLIONSKY, D.J. The Atg8 and Atg12 ubiquitin-like conjugation systems in macroautophagy**. EMBO reports**, v. 9, n. 9, p. 859-864, 2008.

GINGRAS, A. C.; RAUGHT, B.; SONENBERG, N. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. **Annual review of biochemistry**, v. 68, n. 1, p. 913-963, 1999.

GRANT, B. D.; DONALDSON, J. G. Pathways and mechanisms of endocytic recycling. **Nature reviews Molecular cell biology**, v. 10, n. 9, p. 597-608, 2009.

GRATTA, F. *et al*. Effect of feed restriction timing on live performance, breast myopathy occurrence, and muscle fiber degeneration in 2 broiler chicken genetic lines. **Poultry Science**, v. 98, n. 11, p. 5465-5476, 2019.

GRECO, S. *et al*. Common micro-RNA signature in skeletal muscle damage and regeneration induced by Duchenne muscular dystrophy and acute ischemia. **The FASEB Journal**, v. 23, n. 10, p. 3335-3346, 2009.

GRIFFIN, J. *et al.* Transcriptional comparison of myogenesis in leghorn and low score normal embryos. **Poultry science**, v. 96, n. 6, p. 1531-1543, 2017.

GRIFFITHS-JONES, S. *et al.* miRBase: microRNA sequences, targets and gene nomenclature**. Nucleic acids researc**h, v. 34, n. 1, p. 140-144, 2006.

HAFNER, A. *et al*. The multiple mechanisms that regulate p53 activity and cell fate. **Nature reviews Molecular cell biology**, v. 20, n. 4, p. 199-210, 2019.

HAY, N. S. N. Upstream and downstream of *mTOR*. **Genes Development**, v. 18, p. 1926-1945, 2004.

HE, M. X.; HE, Y. W. *CFLAR*/c-FLIPL: a star in the autophagy, apoptosis and necroptosis alliance. **Autophagy**, v. 9, n. 5, p. 791-793, 2013.

HENNING, R. H.; BRUNDEL, B. J. J. M. Proteostasis in cardiac health and disease. **Nature Reviews Cardiology**, v. 14, n. 11, p. 637-653, 2017.

HERSHKO, A.; CIECHANOVER, A.. The ubiquitin system. **Annual review of biochemistry**, v. 67, n. 1, p. 425-479, 1998.

HETZ, C.; CHEVET, Eric; OAKES, Scott A. Proteostasis control by the unfolded protein response. **Nature cell biology**, v. 17, n. 7, p. 829-838, 2015.

HODSON, N. *et al*. Differential localization and anabolic responsiveness of *mTOR* complexes in human skeletal muscle in response to feeding and exercise. **American Journal of Physiology-Cell Physiology**, v. 313, n. 6, p. C604-C611, 2017.

HOLBRO, T.; CIVENNI, G.; HYNES, N. E. The *ERBB* receptors and their role in cancer progression. **The EGF Receptor Family**, p. 103-114, 2003.

HOSAKA, T. *et al*. Disruption of forkhead transcription factor (*FOXO*) family members in mice reveals their functional diversification. **Proceedings of the National Academy of Sciences**, v. 101, n. 9, p. 2975-2980, 2004.

HOSOTANI, M. *et al*. Physiological and pathological mitochondrial clearance is related to pectoralis major muscle pathogenesis in broilers with *Wooden Breast* syndrome. **Frontiers in physiology**, v. 11, p. 579, 2020.

HOTAMISLIGIL, G. S. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. **Cell**, v. 140, n. 6, p. 900-917, 2010.

HOVING-BOLINK, R. A. H. *et al.* Fibre area and capillary supply in broiler breast muscle in relation to productivity and ascites. **Meat science**, v. 56, n. 4, p. 397-402, 2000.

HSU, S. D. *et al.* miRTarBase update 2014: an information resource for experimentally validated miRNA-target interactions. **Nucleic acids research**, v. 42, n. 1, p. 78-85, 2014.

HUTVÁGNER, G.; ZAMORE, P. D. A microRNA in a multiple-turnover RNAi enzyme complex. **Science**, v. 297, n. 5589, p. 2056-2060, 2002.

HYNES, N. E.; LANE, H. A. *ERBB* receptors and cancer: the complexity of targeted inhibitors. **Nature Reviews Cancer**, v. 5, n. 5, p. 341-354, 2005.

IBGE. **Instituto Brasileiro de Geografia e Estatística**. Brasil 2022. Disponível em: <https://www.ibge.gov.br/apps/populacao/projecao/index.html> Acesso em: 24 jun. 2022.

IBRAHIM, D. *et al*. Promising role of growth hormone-boosting peptide in regulating the expression of muscle-specific genes and related micrornas in broiler chickens. **Animals**, v. 11, n. 7, p. 1906, 2021.

INOKI, K. *et al*. TSC2 is phosphorylated and inhibited by Akt and suppresses *mTOR* signalling. **Nature cell biology**, v. 4, n. 9, p. 648-657, 2002.

IYENGAR, P. V. Regulation of Ubiquitin Enzymes in the *TGF-β* Pathway. **International journal of molecular sciences**, v. 18, n. 4, p. 877, 2017.

JAMES, D. E.; JENKINS, A. B.; KRAEGEN, E. W. Heterogeneity of insulin action in individual muscles in vivo: euglycemic clamp studies in rats. American **Journal of Physiology-Endocrinology And Metabolism**, v. 248, n. 5, p. E567-E574, 1985.

JAN, C. H.; WILLIAMS, C. C.; WEISSMAN, J. S. Principles of ER cotranslational translocation revealed by proximity-specific ribosome profiling. **Science**, v. 346, n. 6210, p. 1257521, 2014.

JANASZAK-JASIECKA, A. *et al*. miR-429 regulates the transition between Hypoxia-Inducible Factor (HIF) 1A and HIF3A expression in human endothelial cells. **Scientific reports**, v. 6, n. 1, p. 1-12, 2016.

JOHNSON, C. D. *et al*. The let-7 microRNA represses cell proliferation pathways in human cells. **Cancer research**, v. 67, n. 16, p. 7713-7722, 2007.

JOINER, K.S. *et al.* Evaluation of capillary and myofiber density in the pectoralis major muscles of rapidly growing, high-yield broiler chickens during increased heat stress. **Avian diseases**, v. 58, n. 3, p. 377-382, 2014.

JULIAN, R. J. Rapid growth problems: ascites and skeletal deformities in broilers**.** **Poultry science**, v. 77, n. 12, p. 1773-1780, 1998.

KADOWAKI, H.; NISHITOH, H. Endoplasmic reticulum quality control by garbage disposal. **The FEBS Journal,** v. 286, n. 2, p. 232-240, 2019.

KAJIMOTO, K.; NARABA, H.; IWAI, N. MicroRNA and 3T3-L1 pre-adipocyte differentiation. **RNA**, v. 12, n. 9, p. 1626-1632, 2006.

KATO, Y. *et al*. Real-time functional imaging for monitoring miR-133 during myogenic differentiation. **The international journal of biochemistry & cell biology**, v. 41, n. 11, p. 2225-2231, 2009.

KEGG. Pathway: gga01100, Metabolic pathways - Gallus gallus (chicken). Disponível em: <https://www.genome.jp/entry/pathway+gga01100> Acesso em: 02 jan. 2023.

KHALIL, R. Ubiquitin-proteasome pathway and muscle atrophy. **Muscle atrophy**, p. 235-248, 2018.

KITAJIMA, Y. *et al*. Proteasome dysfunction induces muscle growth defects and protein aggregation. **Journal of cell science,** v. 127, n. 24, p. 5204-5217, 2014.

KITAJIMA, Y.; YOSHIOKA, K.; SUZUKI, N. The ubiquitin–proteasome system in regulation of the skeletal muscle homeostasis and atrophy: from basic science to disorders. **The Journal of Physiological Sciences**, v. 70, n. 1, p. 1-12, 2020.

KLIONSKY, D. J. *et al*. Autophagy in major human diseases. **The EMBO journal**, v. 40, n. 19, p. e108863, 2021.

KLIONSKY, D. J.; EMR, S. D. Autophagy as a regulated pathway of cellular degradation. **Science**, v. 290, n. 5497, p. 1717-1721, 2000.

KONG, D. *et al*. miR-200 regulates PDGF-D-mediated epithelial–mesenchymal transition, adhesion, and invasion of prostate cancer cells. **Stem cells**, v. 27, n. 8, p. 1712-1721, 2009.

KOZOMARA, A.; GRIFFITHS-JONES, S. miRBase: annotating high confidence microRNAs using deep sequencing data. **Nucleic acids research**, v. 42, n. D1, p. D68-D73, 2014.

KUTTAPPAN, V. A. *et al.* Pathological changes associated with *White Striping* in broiler breast muscles. **Poultry Science**, v. 92, n. 2, p. 331-338, 2013a.

KUTTAPPAN, V. A. *et al.* Estimation of factors associated with the occurrence of *White Striping* in broiler breast fillets. **Poultry science**, v. 92, n. 3, p. 811-819, 2013b.

KUTTAPPAN, V. A. *et al.* Consumer acceptance of visual appearance of broiler breast meat with varying degrees of *White Striping***. Poultry Science**, v. 91, n. 5, p. 1240-1247, 2012a.

KUTTAPPAN, V. A. *et al.* Influence of growth rate on the occurrence of *White Striping* in broiler breast fillets. **Poultry Science**, v. 91, n. 10, p. 2677-2685, 2012b.

KUTTAPPAN, V. A. *et al.* Incidence of broiler breast myopathies at 2 different ages and its impact on selected raw meat quality parameters. **Poultry Science**, v. 96, n. 8, p. 3005-3009, 2017.

KUTTAPPAN, V. A.; HARGIS, B. M.; OWENS, C. M. *White Striping* and woody breast myopathies in the modern poultry industry: a review. **Poultry Science**, v. 95, n. 11, p. 2724-2733, 2016.

LAKE, J. A. *et al*. Identification of circulating metabolites associated with *Wooden Breast* and *White Striping*. **Plos one**, v. 17, n. 9, p. e0274208, 2022.

LAKE, J. A.; DEKKERS, J.; ABASHT, B. Genetic basis and identification of candidate genes for *Wooden Breast* and *White Striping* in commercial broiler chickens. **Scientific Reports**, v. 11, n. 1, p. 1-13, 2021.

LEE, S.; DONG, H. H. *FoxO* integration of insulin signaling with glucose and lipid metabolism. **The Journal of endocrinology**, v. 233, n. 2, p. R67, 2017.

LEE, R. C.; FEINBAUM, R. L.; AMBROS, V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. **Cell**, v. 75, n. 5, p. 843-854, 1993.

LÉGER, B. *et al*. Akt signalling through GSK-3β, *mTOR* and *FoxO*1 is involved in human skeletal muscle hypertrophy and atrophy. **The Journal of physiology**, v. 576, n. 3, p. 923-933, 2006.

LEVINE, B.; KROEMER, G. Autophagy in the pathogenesis of disease. **Cell**, v. 132, n. 1, p. 27-42, 2008.

LI, C. *et al*. MicroRNA-29b-2-5p inhibits cell proliferation by directly targeting Cbl-b in pancreatic ductal adenocarcinoma. **BMC cancer**, v. 18, n. 1, p. 1-14, 2018.

LI, C. K. *et al*. Overexpression of MHC class I heavy chain protein in young skeletal muscle leads to severe myositis: implications for juvenile myositis. **The American journal of pathology**, v. 175, n. 3, p. 1030-1040, 2009.

LI, L.; JIA, L.; DING, Y. Upregulation of miR‐375 inhibits human liver cancer cell growth by modulating cell proliferation and apoptosis via targeting *ERBB*2. **Oncology Letters**, v. 16, n. 3, p. 3319-3326, 2018.

LI, X. *et al*. MicroRNA-30d regulates cardiomyocyte pyroptosis by directly targeting *FoxO*3a in diabetic cardiomyopathy. **Cell death & disease**, v. 5, n. 10, p. e1479-e1479, 2014.

LI, S. *et al*. MicroRNAs inhibit the translation of target mRNAs on the endoplasmic reticulum in Arabidopsis. **Cell**, v. 153, n. 3, p. 562-574, 2013.

LIN, X. M.; CHEN, H.; ZHAN, X. L. MiR-203 regulates JAK-STAT pathway in affecting pancreatic cancer cells proliferation and apoptosis by targeting SOCS3. **Eur Rev Med Pharmacol Sci**, v. 23, n. 16, p. 6906-6913, 2019.

LIU, W. M. *et al.* Sperm-borne microRNA-34c is required for the first cleavage division in mouse. **Proceedings of the National Academy of Sciences**, v. 109, n. 2, p. 490-494, 2012.

LIU, Y. *et al*. MiR-375: A novel multifunctional regulator. **Life Sciences**, v. 275, p. 119323, 2021.

LIU, Y. *et al*. miRNA–mRNA network regulation in the skeletal muscle fiber phenotype of chickens revealed by integrated analysis of miRNAome and transcriptome. **Scientific reports**, v. 10, n. 1, p. 1-9, 2020.

LIVINGSTON, M. L. *et al*. *White Striping* and *Wooden Breast* myopathies of broiler breast muscle is affected by time-limited feeding, genetic background, and egg storage. **Poultry science**, v. 98, n. 1, p. 217-226, 2019.

LORENZI, M. *et al*. Incidence of *White Striping* under commercial conditions in medium and heavy broiler chickens in Italy. **Journal of Applied Poultry Research**, v. 23, n. 4, p. 754-758, 2014.

LUNDBERG, J. O.; WEITZBERG, E. Nitrite reduction to nitric oxide in the vasculature. **American Journal of Physiology-Heart and Circulatory Physiology,** v. 295, n. 2, p. 477-478, 2008.

LUZIO, J. P.; PRYOR, P. R.; BRIGHT, N. A. Lysosomes: fusion and function. **Nature reviews Molecular cell biology**, v. 8, n. 8, p. 622-632, 2007.

MA, X.; MA, C.; ZHENG, X. MicroRNA-155 in the pathogenesis of atherosclerosis: a conflicting role?. **Heart, Lung and Circulation**, v. 22, n. 10, p. 811-818, 2013.

MAHARJAN, P. *et al*. *Woody breast* myopathy broiler show age-dependent adaptive differential gene expression in Pectoralis major and altered in-vivo triglyceride kinetics in adipogenic tissues. **Poultry science**, v. 100, n. 7, p. 101092, 2021.

MALILA, Y. *et al*. Differential expression patterns of genes associated with metabolisms, muscle growth and repair in Pectoralis major muscles of fast-and medium-growing chickens. **Plos one**, v. 17, n. 10, p. e0275160, 2022.

MALILA, Y. *et al*. Insights Into Transcriptome Profiles Associated With *Wooden Breast* Myopathy in Broilers Slaughtered at the Age of 6 or 7 Weeks. **Frontiers in Physiology**, p. 996, 2021.

MARCHESI, J. A. P. *et al.* Whole transcriptome analysis of the pectoralis major muscle reveals molecular mechanisms involved with *White Striping* in broiler chickens. **Poultry science**, v. 98, n. 2, p. 590-601, 2019.

MARCIANO, C. M. M. *et al*. Differential Expression of Myogenic and Calcium Signaling-Related Genes in Broilers Affected With *White Striping*. **Frontiers in Physiology**, v. 12, 2021.

MARKWORTH, J. F. *et al*. Ibuprofen treatment blunts early translational signaling responses in human skeletal muscle following resistance exercise. **Journal of applied physiology**, v. 117, n. 1, p. 20-28, 2014.

MARTIN-ALONSO, A. *et al*. Differentially expressed microRNAs in experimental cerebral malaria and their involvement in endocytosis, adherens junctions, *FoxO* and TGF-β signalling pathways. **Scientific reports**, v. 8, n. 1, p. 1-10, 2018.

MCCARTHY, D, J.; CHEN, Y.; SMYTH, G. K. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. **Nucleic Acids Research**, v. 40, n. 10, p. 4288-4297, 2012.

MCMURTRY, J. P.; FRANCIS, G. L.; UPTON, Z. Insulin-like growth factors in poultry. **Domestic animal endocrinology**, v. 14, n. 4, p. 199-229, 1997.

MISKA, E. A.; FERGUSON-SMITH, A. C. Transgenerational inheritance: models and mechanisms of non–DNA sequence–based inheritance. **Science**, v. 354, n. 6308, p. 59-63, 2016.

MIZUSHIMA, N. *et al*. Autophagy fights disease through cellular self-digestion. **Nature**, v. 451, n. 7182, p. 1069-1075, 2008.

MIZUSHIMA, N.; KLIONSKY, D. J. Protein turnover via autophagy: implications for metabolism. **Annual review of nutrition**, v. 27, n. 1, p. 19-40, 2007.

MIZUSHIMA, N.; KOMATSU, M.. Autophagy: renovation of cells and tissues. **Cell**, v. 147, n. 4, p. 728-741, 2011.

MOURELATOS, Z. *et al.* miRNPs: a novel class of ribonucleoproteins containing numerous microRNAs. **Genes & development**, v. 16, n. 6, p. 720-728, 2002.

MUDALAL, S. *et al.* Quantity and functionality of protein fractions in chicken breast fillets affected by *White Striping*. **Poultry science**, v. 93, n. 8, p. 2108-2116, 2014.

MUELLER, N. *et al*. De novo mutation in ELOVL1 causes ichthyosis, acanthosis nigricans, hypomyelination, spastic paraplegia, high frequency deafness and optic atrophy. **Journal of medical genetics**, v. 56, n. 3, p. 164-175, 2019.

MURPHY, C. T.; HU, P. J. Insulin/insulin-like growth factor signaling in C. elegans. WormBook: The Online Review of C. elegans. **Biology**, 2018.

MUTRYN, M. F. *et al.* Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing. **BMC genomics**, v. 16, n. 1, p. 1-19, 2015.

NAKANISHI, K.; SUDO, T.; MORISHIMA, N.. Endoplasmic reticulum stress signaling transmitted by ATF6 mediates apoptosis during muscle development. **The Journal of cell biology**, v. 169, n. 4, p. 555-560, 2005.

NAKATOGAWA, H. Mechanisms governing autophagosome biogenesis. **Nature reviews Molecular cell biology**, v. 21, n. 8, p. 439-458, 2020.

NAZARI, N. *et al*. The emerging role of microRNA in regulating the *mTOR* signaling pathway in immune and inflammatory responses. **Immunology and Cell Biology**, v. 99, n. 8, p. 814-832, 2021.

NEMATBAKHSH, S. *et al*. Molecular regulation of lipogenesis, adipogenesis and fat deposition in chicken. **Genes**, v. 12, n. 3, p. 414, 2021.

NIE, M. *et al*. Insights Into miRNA-mRNA Regulatory Mechanisms of Cold Adaptation in Gymnocypris eckloni: Ubiquitin-Mediated Proteolysis Is Pivotal for Adaptive Energy Metabolism. **Frontiers in Genetics**, v. 13, 2022.

NORRING, M. *et al. Wooden Breast* myopathy links with poorer gait in broiler chickens. **Animals**, v. 13, n. 8, p. 1690-1695, 2019.

OLIVEIRA, R. F. *et al*. Effect of aging on the quality of breast meat from broilers affected by *Wooden Breast* myopathy. **Animals**, v. 11, n. 7, p. 1960, 2021.

ONO, K. *et al*. Identification of microRNA that represses IRS-1 expression in liver. **PLoS One**, v. 13, n. 1, p. e0191553, 2018.

OUYANG, H. *et al*. Deep sequencing analysis of miRNA expression in breast muscle of fast-growing and slow-growing broilers. **International journal of molecular sciences**, v. 16, n. 7, p. 16242-16262, 2015.

PAGANO, T. B. *et al*. Age related skeletal muscle atrophy and upregulation of autophagy in dogs. **The Veterinary Journal**, v. 206, n. 1, p. 54-60, 2015.

PAJCINI, K. V. *et al*. Myoblasts and macrophages share molecular components that contribute to cell–cell fusion**. The Journal of cell biology**, v. 180, n. 5, p. 1005-1019, 2008.

PAMPOUILLE, E. *et al.* Mapping QTL for *White Striping* in relation to breast muscle yield and meat quality traits in broiler chickens. **BMC genomics**, v. 19, n. 1, p. 1-14, 2018.

PAPAH, M. B. *et al*. Gene expression profiling of the early pathogenesis of *Wooden Breast* disease in commercial broiler chickens using RNA-sequencing. **PLoS One**, v. 13, n. 12, p. e0207346, 2018.

PAPAH, M.B.; ABASHT, B. Dysregulation of lipid metabolism and appearance of slow myofiber-specific isoforms accompany the development of *Wooden Breast* myopathy in modern broiler chickens. **Scientific Reports**, v. 9, n. 1, p. 1-12, 2019.

PARKER, M. I. *et al*. Proliferative signaling by *ERBB* proteins and RAF/MEK/ERK effectors in polycystic kidney disease. **Cellular signalling**, v. 67, p. 109497, 2020.

PARKES, J. E. *et al*. The role of microRNAs in the idiopathic inflammatory myopathies. **Current opinion in rheumatology**, v. 27, n. 6, p. 608-615, 2015.

PATERSON, A. D. *et al*. Characterization of E-cadherin endocytosis in isolated MCF-7 and chinese hamster ovary cells: the initial fate of unbound E-cadherin. **Journal of Biological Chemistry**, v. 278, n. 23, p. 21050-21057, 2003.

PERLUIGI, M.; DI DOMENICO, F.; BUTTERFIELD, D. A. *mTOR* signaling in aging and neurodegeneration: at the crossroad between metabolism dysfunction and impairment of autophagy**. Neurobiology of disease**, v. 84, p. 39-49, 2015.

PETERSEN, K. Falk; SHULMAN, G. I. Etiology of insulin resistance. The **American journal of medicine**, v. 119, n. 5, p. S10-S16, 2006.

PETRACCI, M. *et al.* Meat quality in fast-growing broiler chickens. World's **Poultry Science Journal**, v. 71, n. 2, p. 363-374, 2015.

PETRACCI, M.; CAVANI, C. Muscle growth and poultry meat quality issues. **Nutrients**, v. 4, n. 1, p. 1-12, 2012.

PETRACCI, M. *et al.* Occurrence of *White Striping* under commercial conditions and its impact on breast meat quality in broiler chickens. **Poultry Science**, v. 92, n. 6, p. 1670-1675, 2013.

PICKART, C. M. Mechanisms underlying ubiquitination. **Annual review of biochemistry**, v. 70, 2001.

PILLAY, C. S.; ELLIOTT, E.; DENNISON, C. **Endolysosomal proteolysis and its regulation. Biochemical** Journal, v. 363, n. 3, p. 417-429, 2002.

POON, R. Y. C. Cell cycle control: a system of interlinking oscillators. **Cell Cycle Oscillators**, p. 3-19, 2016.

POTENTE, M. *et al*. Involvement of *FoxO* transcription factors in angiogenesis and postnatal neovascularization. **The Journal of clinical investigation**, v. 115, n. 9, p. 2382-2392, 2005.

PRAUD, C. *et al.* Molecular phenotyping of *White Striping* and *Wooden Breast* myopathies in chicken. **Frontiers in Physiology**, v. 11, p. 633, 2020.

PRICE, N. L. *et al*. Genetic ablation of miR-33 increases food intake, enhances adipose tissue expansion, and promotes obesity and insulin resistance**. Cell reports**, v. 22, n. 8, p. 2133-2145, 2018.

PRISCO, F. *et al*. Pathologic characterization of *White Striping* myopathy in broiler chickens. **Poultry Science**, v. 100, n. 7, p. 101150, 2021.

PUIG, O.; TJIAN, R. Nutrient availability and growth: regulation of insulin signaling by d*FOXO*/*FOXO*1. **Cell cycle**, v. 5, n. 5, p. 503-505, 2006.

RABINOWITZ, J. D.; WHITE, E. Autophagy and metabolism. **Science**, v. 330, n. 6009, p. 1344-1348, 2010.

RACANELLI, A.C *et al*. Autophagy and inflammation in chronic respiratory disease**. Autophagy**, v. 14, n. 2, p. 221-232, 2018.

RAINS, J. L.; JAIN, S. K. Oxidative stress, insulin signaling, and diabetes. Free Radical Biology and Medicine, v. 50, n. 5, p. 567-575, 2011.

RAJAGOPAL, S.; SHENOY, S. K. GPCR desensitization: Acute and prolonged phases. **Cellular signalling**, v. 41, p. 9-16, 2018.

RASSOULZADEGAN, M. *et al.* RNA-mediated non-mendelian inheritance of an epigenetic change in the mouse. **Nature**, v. 441, n. 7092, p. 469-474, 2006.

RAYAVARAPU, S.; COLEY, W.; NAGARAJU, K. Endoplasmic reticulum stress in skeletal muscle homeostasis and disease. **Current rheumatology reports**, v. 14, n. 3, p. 238-243, 2012.

REID, D. W.; NICCHITTA, C. V. Diversity and selectivity in mRNA translation on the endoplasmic reticulum. **Nature reviews Molecular cell biology**, v. 16, n. 4, p. 221-231, 2015.

RELAIX, F; ZAMMIT, P. S. Satellite cells are essential for skeletal muscle regeneration: the cell on the edge returns center stage. **Development**, v. 139, n. 16, p. 2845-2856, 2012.

ROBINSON, M.D.; MCCARTHY, D. J.; SMYTH, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. **Bioinformatics**, v. 26, n. 1, p. 139-140, 2010.

RODGERS, A. B. *et al.* Transgenerational epigenetic programming via sperm microRNA recapitulates effects of paternal stress. **Proceedings of the National Academy of Sciences**, v. 112, n. 44, p. 13699-13704, 2015.

RONNEBAUM, S. M.; PATTERSON, Cam. The *FoxO* family in cardiac function and dysfunction. **Annual review of physiology**, v. 72, p. 81, 2010.

RUEDA, A. *et al*. sRNAtoolbox: an integrated collection of small RNA research tools. Nucleic acids research, v. 43, n. W1, p. W467-W473, 2015.

RUGGIANO, A.; FORESTI, O.; CARVALHO, P. ER-associated degradation: Protein quality control and beyond**. Journal of Cell Biology**, v. 204, n. 6, p. 869-879, 2014.

RUSSELL, R. C.; YUAN, H. X.; GUAN, K. L. Autophagy regulation by nutrient signaling. **Cell research**, v. 24, n. 1, p. 42-57, 2014.

RUSSO, E. *et al*. Evaluation of *White Striping* prevalence and predisposing factors in broilers at slaughter. **Poultry science**, v. 94, n. 8, p. 1843-1848, 2015.

RYTER, S. W. *et al*. Mechanisms of cell death in oxidative stress. **Antioxidants & redox signaling**, v. 9, n. 1, p. 49-89, 2007.

SALCINI, A. E.*et al*. Epidermal growth factor pathway substrate 15, Eps15. **The international journal of biochemistry & cell biology**, v. 31, n. 8, p. 805-809, 1999.

SALIMINEJAD, K. *et al*. An overview of microRNAs: biology, functions, therapeutics, and analysis methods. **Journal of cellular physiology**, v. 234, n. 5, p. 5451-5465, 2019.

SCHEELE, C. W. Pathological changes in metabolism of poultry related to increasing production levels. **Veterinary Quarterly**, v. 19, n. 3, p. 127-130, 1997.

SCHWARZ, D. S.; BLOWER, M. D. The endoplasmic reticulum: structure, function and response to cellular signaling. Cellular and molecular life sciences, v. 73, n. 1, p. 79-94, 2016.

SHU, J. *et al*. Deep sequencing microRNA profiles associated with *Wooden Breast* in commercial broilers. **Poultry Science**, v. 100, n. 12, p. 101496, 2021.

SIHVO, H. K.; IMMONEN, K.; PUOLANNE, E. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. **Veterinary pathology**, v. 51, n. 3, p. 619-623, 2014.

SINGH, G. B.; COWAN, D. B.; WANG, D. Z. Tiny regulators of massive tissue: microRNAs in skeletal muscle development, myopathies, and cancer cachexia. **Frontiers in Oncology,** v. 10, p. 598964, 2020.

SKVORTSOVA, K.; IOVINO, N.; BOGDANOVIĆ, O. Functions and mechanisms of epigenetic inheritance in animals**. Nature reviews Molecular cell biology**, v. 19, n. 12, p. 774-790, 2018.

SMITH, T.; HEGER, A.; SUDBERY, I. UMI-tools: modeling sequencing errors in Unique Molecular Identifiers to improve quantification accuracy. **Genome research**, v. 27, n. 3, p. 491-499, 2017.

SOBAH, M. L.; LIONGUE, C.; WARD, A. C. SOCS proteins in immunity, inflammatory diseases, and immune-related cancer. **Frontiers in Medicine**, p. 1532, 2021.

SOGLIA, F. *et al*. A critical review of the mechanisms involved in the occurrence of growth-related abnormalities affecting broiler chicken breast muscles. **Poultry Science**, v. 100, n. 6, p. 101180, 2021.

SOGLIA, F.*et al.* Superficial and deep changes of histology, texture and particle size distribution in broiler *Wooden Breast* muscle during refrigerated storage. **Poultry Science**, v. 96, n. 9, p. 3465-3472, 2017.

STEINMAN, R. M. *et al*. Endocytosis and the recycling of plasma membrane. **The Journal of cell biology**, v. 96, n. 1, p. 1-27, 1983.

SUN, X. *et al*. Knockdown of lncRNA XIST suppresses osteosarcoma progression by inactivating AKT/*mTOR* signaling pathway by sponging miR-375-3p. **International Journal of Clinical and Experimental Pathology**, v. 12, n. 5, p. 1507, 2019.

TAETZSCH, T. *et al*. The microRNA miR-133b functions to slow Duchenne muscular dystrophy pathogenesis. **The Journal of Physiology**, v. 599, n. 1, p. 171-192, 2021.

TANG, F.*et al.* Maternal microRNAs are essential for mouse zygotic development. **Genes & development**, v. 21, n. 6, p. 644-648, 2007.

TANG, R.; LANGDON, W. Y.; ZHANG, J. Regulation of immune responses by E3 ubiquitin ligase Cbl-b. **Cellular immunology**, v. 340, p. 103878, 2019.

TANG, X.g *et al*. Identification of glucose-regulated miRNAs from pancreatic β cells reveals a role for miR-30d in insulin transcription. **RNA**, v. 15, n. 2, p. 287-293, 2009.

TARGETSCAN, **Chicken ORC genes**. Disponível em: <https://www.targetscan.org/cgi-bin/targetscan/vert_80/view_gene.cgi?rs=ENST00000392857.5&taxid=9031&showcnc=0&shownc=0&shownc_nc=&showncf1=&showncf2=&subset=1> . Acesso em: 15 de jan. 2023a.

TIJARE, V. V. *et al*. Meat quality of broiler breast fillets with *White Striping* and woody breast muscle myopathies. **Poultry Science**, v. 95, n. 9, p. 2167-2173, 2016.

TROCINO, A. *et al.* Effect of genotype, gender and feed restriction on growth, meat quality and the occurrence of Shite Striping and *Wooden Breast* in broiler chickens. **Poultry science**, v. 94, n. 12, p. 2996-3004, 2015.

TSAI, W. C. *et al*. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. **The Journal of clinical investigation**, v. 122, n. 8, p. 2884-2897, 2012.

TURNER, N. *et al*. Fatty acid metabolism, energy expenditure and insulin resistance in muscle. **Journal of Endocrinology**, v. 220, n. 2, p. T61-T79, 2014.

TUSCHL, T. *et al.* Targeted mRNA degradation by double-stranded RNA in vitro. **Genes & development**, v. 13, n. 24, p. 3191-3197, 1999.

TZAHAR, E. *et al*. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. **Molecular and cellular biology**, v. 16, n. 10, p. 5276-5287, 1996.

UCAR, A. *et al*. The miRNA-212/132 family regulates both cardiac hypertrophy and cardiomyocyte autophagy. **Nature communications**, v. 3, n. 1, p. 1-11, 2012.

VELLEMAN, S. G.; COY, C. S.; ABASHT, B.. Effect of expression of PPARG, DNM2L, RRAD, and LINGO1 on broiler chicken breast muscle satellite cell function. **Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology**, v. 268, p. 111-186, 2022.

VITADELLO, M. *et al*. Myofiber stress-response in myositis: parallel investigations on patients and experimental animal models of muscle regeneration and systemic inflammation**. Arthritis research & therapy**, v. 12, n. 2, p. 1-15, 2010.

WAGNER, J. *et al*. Characterization of levels and cellular transfer of circulating lipoprotein-bound microRNAs. **Arteriosclerosis, thrombosis, and vascular biology**, v. 33, n. 6, p. 1392-1400, 2013.

WALTER, P.; RON, D. The unfolded protein response: from stress pathway to homeostatic regulation. **Science**, v. 334, n. 6059, p. 1081-1086, 2011.

WAN, J.*et al*. Inhibition of miR-155 reduces impaired autophagy and improves prognosis in an experimental pancreatitis mouse model. **Cell death & disease**, v. 10, n. 4, p. 1-11, 2019.

WANG, J. *et al*. Alpha-tocopherol acetate and alpha lipoic acid may mitigate the development of *Wooden Breast* myopathy in broilers at an early age. British **Poultry Science**, v. 62, n. 5, p. 749-758, 2021.

WANG, J. *et al*. Effects of microRNAs on skeletal muscle development. **Gene**, v. 668, p. 107-113, 2018.

WANG, Y.; LEE, C. G. L. MicroRNA and cancer–focus on apoptosis. **Journal of cellular and molecular medicine**, v. 13, n. 1, p. 12-23, 2009.

WIGHTMAN, B.; HA, I.; RUVKUN, G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. **Cell**, v. 75, n. 5, p. 855-862, 1993.

WILLIAMS, A. H. *et al*. MicroRNA control of muscle development and disease. **Current opinion in cell biology**, v. 21, n. 3, p. 461-469, 2009.

WOLFRUM, C. *et al*. Insulin regulates the activity of forkhead transcription factor Hnf-3β/Foxa-2 by Akt-mediated phosphorylation and nuclear/cytosolic localization. **Proceedings of the National Academy of Sciences**, v. 100, n. 20, p. 11624-11629, 2003.

WULLSCHLEGER, S.; LOEWITH, R.; HALL, M. N. TOR signaling in growth and metabolism. **Cell**, v. 124, n. 3, p. 471-484, 2006.

XING, T. *et al*. Hepatic oxidative stress, apoptosis, and inflammation in broiler chickens with *Wooden Breast* myopathy. **Frontiers in physiology**, p. 415, 2021.

YAN, W. *et al*. Cancer-cell-secreted miR-122 suppresses O-GlcNAcylation to promote skeletal muscle proteolysis. Nature cell biology, v. 24, n. 5, p. 793-804, 2022.

YANG, W. M. *et al*. Obesity-induced miR-15b is linked causally to the development of insulin resistance through the repression of the insulin receptor in hepatocytes. **Molecular nutrition & food research**, v. 59, n. 11, p. 2303-2314, 2015.

YARDEN, Y.; SLIWKOWSKI, M.X. Untangling the *ERBB* signalling network. **Nature reviews Molecular cell biology**, v. 2, n. 2, p. 127-137, 2001.

YE, X.; ZHOU, X. J.; ZHANG, H. Exploring the role of autophagy-related gene 5 (*ATG5*) yields important insights into autophagy in autoimmune/autoinflammatory diseases. **Frontiers in immunology**, v. 9, p. 2334, 2018.

YIN, H. *et al*. miR-9-5p inhibits skeletal muscle satellite cell proliferation and differentiation by targeting IGF2BP3 through the IGF2-PI3K/Akt signaling pathway. **International journal of molecular sciences**, v. 21, n. 5, p. 1655, 2020.

YUAN, K. T. *et al*. Deregulation of MicroRNA-375 inhibits proliferation and migration in gastric cancer in association with autophagy-mediated AKT/*mTOR* signaling pathways. **Technology in Cancer Research & Treatment**, v. 17, p. 153, 2018.

ZAMBONELLI, P. *et al*. Detection of differentially expressed genes in broiler pectoralis major muscle affected by *White Striping–Wooden Breast* myopathies. **Poultry Science**, v. 95, n. 12, p. 2771-2785, 2016.

ZAMORE, P. D. *et al.* RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. **Cell**, v. 101, n. 1, p. 25-33, 2000.

ZANETTI, M. A. *et al*. Economic losses associated with *Wooden Breast* and *White Striping* in broilers. **Seminário: Ciências Agrárias**, v. 39, n. 2, p. 887-891, 2018.

ZHANG, L. *et al*. A systematic analysis on mRNA and microRNA expression in runting and stunting chickens. **PloS one**, v. 10, n. 5, p. e0127342, 2015.

ZUIDHOF, M. J. *et al*. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. **Poultry science**, v. 93, n. 12, p. 2970-2982, 2014

6 ANEXOS

6.1 ANEXO A – COMPROVANTE DO CEUA

Embrapa	Certificado*	ETICA 1/1

**Em concordância com a Orientação Técnica CONCEA no 8, de 18 de março de 2016 (Anexo I)*

Certificamos que a proposta intitulada **"Prospecção de genes e vias metabólicas envolvidas na manifestação de miopatias peitorais em frangos de corte"**, registrada com o nº **008/2019**, sob a responsabilidade de **Jane de Oliveira Peixoto** – que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO(A) Embrapa Suínos e Aves, em reunião de **01/08/2019**.

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência da Autorização	02/02/2020 – 02/02/2023
Espécie/linhagem/raça	*Ave (Animais cativos)/Linha TT e Linhagem comercial Cobb.*
Nº de animais	2000
Peso/Idade	3500.0 kg / 42.0 meses
Sexo	1000 Machos/ 1000 Fêmeas
Origem	Ave (Animais cativos): Granja (Serão usadas da TT no nucleo de conservação do CNPSA e animais comerciais adquiridos de incubatório na região.)

Paulo Augusto Esteves
CRB: 2563603D
Matrícula: 320571

Presidente CEUA/CNPSA

6.2 ANEXO B – TABELAS SUPLEMENTARES

6.2.1 Tabela Suplementar 1 - miRNAs diferencialmente expressos entre frangos afetados com Wooden Breast e frangos do grupo controle

miRNAs	logFC	logCPM	LR	PValue	FDR
gga-miR-146b-5p	1,51	12,31	38,64	5,10E-10	1,55E-07
gga-miR-223	1,93	12,74	34,26	4,81E-09	7,29E-07
gga-miR-146b-3p	1,57	8,01	29,75	4,91E-08	4,96E-06
chr2_9820	-2,01	3,84	26,39	2,79E-07	2,11E-05
gga-miR-155	2,32	7,30	25,45	4,53E-07	2,75E-05
gga-miR-222b-5p	2,61	1,35	24,30	8,26E-07	4,17E-05
gga-miR-222b-3p	2,57	4,81	23,54	1,22E-06	5,30E-05
gga-miR-29a-3p	1,78	10,53	22,74	1,86E-06	6,26E-05
gga-miR-29c-3p	1,78	10,53	22,74	1,86E-06	6,26E-05
gga-miR-29b-3p	2,22	7,22	22,15	2,52E-06	7,27E-05
gga-miR-1a-3p	-1,18	19,12	22,06	2,64E-06	7,27E-05
gga-miR-142-3p	1,74	13,96	21,50	3,54E-06	8,95E-05
gga-miR-133a-3p	-1,02	16,22	20,13	7,22E-06	0,0002
gga-miR-2188-5p	-1,33	8,14	20,20	6,98E-06	0,0002
chr3_13350	-1,17	4,35	19,84	8,42E-06	0,0002
gga-miR-1416-3p	2,54	1,88	18,73	1,50E-05	0,0003
gga-miR-1c	-1,25	7,61	18,34	1,85E-05	0,0003
gga-miR-193a-5p	-1,17	6,88	17,07	3,60E-05	0,0006
gga-miR-365-3p	-0,84	9,14	15,94	6,55E-05	0,0010
gga-miR-1b-3p	-1,01	12,43	15,56	7,97E-05	0,0012
gga-miR-2188-3p	-1,30	2,12	15,27	9,31E-05	0,0013
gga-miR-3530-3p	3,68	0,70	15,13	0,0001	0,0014
gga-miR-133a-5p	-1,05	10,41	14,79	0,0001	0,0016
gga-miR-29a-5p	1,67	3,09	14,56	0,0001	0,0017
gga-miR-1416-5p	1,39	8,83	14,36	0,0002	0,0018
gga-miR-142-5p	1,66	11,08	13,67	0,0002	0,0024
gga-miR-133c-3p	-0,92	14,42	13,74	0,0002	0,0024
gga-miR-12214-5p	2,21	1,15	13,05	0,0003	0,0033
gga-miR-1769-5p	3,70	0,37	12,59	0,0004	0,0034
gga-miR-147	1,71	4,89	12,40	0,0004	0,0034
gga-miR-153-3p	1,44	5,46	12,85	0,0003	0,0034
Gga-Mir-153-P2_3p	1,44	5,46	12,85	0,0003	0,0034

gga-miR-1559-3p	1,27	1,89	12,57	0,0004	0,0034
gga-miR-12243-5p	1,21	3,84	12,53	0,0004	0,0034
gga-miR-1329-5p	1,07	5,44	12,46	0,0004	0,0034
gga-miR-1559-5p	0,93	7,03	12,64	0,0004	0,0034
gga-miR-133b	-0,84	11,14	12,75	0,0004	0,0034
gga-miR-451	-1,25	9,26	12,67	0,0004	0,0034
chr2_9097	-2,72	0,78	12,39	0,0004	0,0034
gga-miR-458a-3p	1,64	6,20	11,98	0,0005	0,0041
gga-miR-144-5p	-1,20	4,92	11,70	0,0006	0,0046
gga-miR-3530-5p	2,56	4,52	11,47	0,0007	0,0050
gga-miR-1a-1-5p	-1,30	5,91	11,46	0,0007	0,0050
gga-miR-1388a-5p	1,05	7,68	11,07	0,0009	0,0059
gga-miR-30b-5p	-0,75	9,39	11,04	0,0009	0,0059
gga-miR-130a-3p	-0,87	6,70	11,02	0,0009	0,0059
gga-miR-205a	3,30	3,51	10,84	0,0010	0,0064
gga-miR-6553-3p	-1,87	3,97	10,24	0,0014	0,0087
chr22_10817	5,52	-0,30	10,09	0,0015	0,0090
gga-miR-30e-5p	-0,55	13,22	10,07	0,0015	0,0090
gga-miR-133c-5p	-1,51	1,41	10,07	0,0015	0,0090
gga-miR-218-5p	0,85	9,22	10,02	0,0015	0,0090
gga-miR-1663-3p	1,44	1,47	9,85	0,0017	0,0095
gga-miR-144-3p	-1,35	5,35	9,85	0,0017	0,0095
gga-miR-1663-5p	2,32	0,14	9,75	0,0018	0,0099
gga-miR-122b-5p	-1,53	1,55	9,67	0,0019	0,0101
gga-miR-1388a-3p	1,20	8,71	9,62	0,0019	0,0102
gga-let-7b	-0,59	12,87	9,34	0,0022	0,0117
gga-miR-132a-5p	1,27	5,20	9,21	0,0024	0,0124
gga-miR-30a-5p	-0,54	12,26	9,10	0,0026	0,0127
gga-miR-1a-2-5p	-1,13	6,24	9,12	0,0025	0,0127
gga-miR-30d	-0,59	12,37	8,35	0,0039	0,0189
gga-miR-29b-1-5p	1,78	0,82	8,19	0,0042	0,0202
gga-miR-203a	1,70	3,13	7,98	0,0047	0,0224
gga-miR-6553-5p	-1,79	1,21	7,58	0,0059	0,0275
gga-miR-1329-3p	1,33	1,29	7,36	0,0067	0,0294
gga-miR-455-5p	0,86	6,02	7,37	0,0066	0,0294
gga-miR-196-5p	-0,65	7,08	7,37	0,0066	0,0294
gga-miR-122-5p	-4,42	9,15	7,35	0,0067	0,0294
gga-miR-132c-5p	1,14	2,71	7,06	0,0079	0,0341
gga-miR-181a-3p	-0,61	5,70	7,04	0,0080	0,0341
gga-miR-30c-5p	-0,50	11,44	6,99	0,0082	0,0346

gga-miR-15b-3p	0,73	2,63	6,76	0,0093	0,0383
gga-miR-30a-3p	-0,56	7,62	6,75	0,0093	0,0383
gga-miR-30c-1-3p	-0,61	6,79	6,71	0,0096	0,0388
gga-miR-12229-5p	-1,23	4,01	6,62	0,0101	0,0402
gga-miR-210a-5p	0,77	8,69	6,56	0,0104	0,0409
gga-miR-148a-5p	0,75	3,77	6,54	0,0106	0,0410
gga-miR-190a-3p	-0,62	3,47	6,47	0,0109	0,0420
gga-miR-132c-3p	1,65	0,41	6,27	0,0123	0,0459
gga-miR-33-2-5p	-0,82	7,01	6,25	0,0124	0,0459
gga-miR-33-5p	-0,82	7,01	6,25	0,0124	0,0459

6.2.2 Tabela Suplementar 2 - miRNAs diferencialmente expressos entre frangos afetados com Wooden Breast e frangos afetados com White Striping

miRNAs	logFC	logCPM	LR	PValue	FDR
gga-miR-1663-5p	4,73	0,14	34,68	3,89E-09	1,18E-06
gga-miR-146b-5p	1,12	12,31	31,77	1,74E-08	2,63E-06
gga-miR-222b-5p	2,12	1,35	28,59	8,96E-08	9,05E-06
gga-miR-223	1,36	12,74	26,60	2,50E-07	1,70E-05
gga-miR-146b-3p	1,20	8,01	26,38	2,80E-07	1,70E-05
gga-miR-29c-3p	1,51	10,53	24,91	6,01E-07	2,60E-05
gga-miR-29a-3p	1,51	10,53	24,91	6,01E-07	2,60E-05
gga-miR-429-3p	3,10	4,25	23,30	1,39E-06	5,25E-05
gga-miR-200b-3p	3,26	4,84	20,02	7,68E-06	0,0003
gga-miR-29b-3p	1,57	7,22	17,67	2,63E-05	0,0007
gga-miR-451	-1,32	9,26	17,84	2,41E-05	0,0007
chr22_10817	5,52	-0,30	17,15	3,45E-05	0,0008
gga-miR-200a-3p	3,12	7,17	17,10	3,55E-05	0,0008
chr2_9820	-1,42	3,84	16,27	5,48E-05	0,0012
gga-miR-365-3p	-0,73	9,14	15,72	7,36E-05	0,0015
gga-miR-155	1,41	7,30	15,19	9,73E-05	0,0018
gga-miR-1416-5p	1,14	8,83	14,48	0,0001	0,0025
gga-miR-2188-5p	-0,99	8,14	14,15	0,0002	0,0028
gga-miR-375	3,22	3,51	13,08	0,0003	0,0043
gga-miR-1663-3p	1,33	1,47	13,15	0,0003	0,0043
gga-miR-142-3p	1,10	13,96	13,25	0,0003	0,0043
gga-miR-147	1,41	4,89	12,98	0,0003	0,0043
gga-miR-193a-3p	-1,25	3,65	12,26	0,0005	0,0061
gga-miR-205a	2,67	3,51	11,78	0,0006	0,0075

gga-miR-133b	-0,70	11,14	11,62	0,0007	0,0079
gga-miR-29a-5p	1,16	3,09	11,17	0,0008	0,0097
gga-miR-222b-3p	1,34	4,81	11,05	0,0009	0,0099
gga-miR-12243-5p	0,91	3,84	10,76	0,0010	0,0110
gga-miR-1559-5p	0,71	7,03	10,72	0,0011	0,0110
gga-miR-458a-3p	1,21	6,20	9,92	0,0016	0,0160
gga-miR-144-3p	-1,21	5,35	9,95	0,0016	0,0160
gga-miR-133a-5p	-0,76	10,41	9,84	0,0017	0,0162
gga-miR-1a-3p	-0,69	19,12	9,57	0,0020	0,0176
gga-miR-193a-5p	-0,77	6,88	9,57	0,0020	0,0176
gga-miR-133a-3p	-0,61	16,22	9,44	0,0021	0,0184
gga-miR-144-5p	-0,93	4,92	9,02	0,0027	0,0218
gga-miR-1a-2-5p	-0,99	6,24	9,02	0,0027	0,0218
gga-miR-1b-3p	-0,67	12,43	8,79	0,0030	0,0241
gga-miR-1416-3p	1,24	1,88	8,74	0,0031	0,0242
gga-miR-142-5p	1,06	11,08	8,47	0,0036	0,0261
gga-miR-33-2-5p	-0,84	7,01	8,48	0,0036	0,0261
gga-miR-33-5p	-0,84	7,01	8,48	0,0036	0,0261
gga-miR-29b-1-5p	1,39	0,82	8,38	0,0038	0,0267
gga-miR-12211-5p	1,34	0,54	8,12	0,0044	0,0301
chr3_13350	-0,66	4,35	8,08	0,0045	0,0301
gga-miR-132a-5p	0,97	5,20	8,02	0,0046	0,0304
gga-miR-133c-3p	-0,62	14,42	7,91	0,0049	0,0310
gga-miR-12229-5p	-1,20	4,01	7,93	0,0049	0,0310
gga-miR-460a-5p	0,91	3,69	7,69	0,0055	0,0343
gga-miR-101-2-5p	-0,63	2,99	7,61	0,0058	0,0351
gga-miR-30e-5p	-0,42	13,22	7,49	0,0062	0,0369
gga-miR-3530-5p	1,59	4,52	7,37	0,0066	0,0382
gga-miR-1329-3p	1,04	1,29	7,36	0,0067	0,0382
gga-miR-1559-3p	0,73	1,89	7,27	0,0070	0,0393
gga-miR-190b-5p	0,55	4,78	7,23	0,0072	0,0395
chr2_9097	-1,89	0,78	7,05	0,0079	0,0430
chr4_15762	3,05	0,15	6,97	0,0083	0,0433
gga-miR-130a-3p	-0,61	6,70	7,00	0,0082	0,0433
gga-miR-1662	0,80	7,28	6,88	0,0087	0,0448
gga-miR-1388a-3p	0,83	8,71	6,82	0,0090	0,0456
gga-miR-1c	-0,67	7,61	6,78	0,0092	0,0457