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Reduction in ribonucleotide reductase subunit RRM2 associated with alterations in mitochondrial proteins, leading to impaired gut health of woody breast chicken

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The woody breast (WB) condition impairs the quality of chicken breast meat, causing a financial loss for the poultry industry. Recent studies suggest that the mitochondria may play a role in muscle and gut health by regulating inflammatory mediators. However, there is limited information available on gut and pancreas health as they relate to WB. Ribonucleotide reductase (RNR), subunit RRM2, is involved in DNA synthesis and mitochondrial function. Inhibition of RRM2 increases gastrointestinal disturbances and apoptosis in the pancreas by disturbing inflammatory mediators and mitochondrial homeostasis. This study aimed to investigate the links between RRM2, pancreas, and gut health in broilers exhibiting WB. Sixty eight-week-old male broilers were used to collect breast muscle, duodenum, and pancreas from 10 broilers exhibiting severe WB and 10 normal (N). Gene expression was measured using qRT-PCR with SYBR reagents. Commercial biochemical assays were used to measure mitochondrial function. To determine if RNR controls mitochondrial functions, an RNR inhibitor was tested *in vitro* on avian muscle-derived cells. Data were analyzed using Prism and *t*-tests. In tissues, RRM2 expression was lower in the muscle (P = 0.03), pancreas, and duodenum (both P = 0.04) vs. N. The expression levels of cytochrome b (Cytb) and mitochondrial uncoupling protein 3 (UCP3) were reduced in the muscle (P = 0.05 and P = 0.002) and pancreas (P = 0.009 and P = 0.002) for WB, while only Cytb was reduced in the duodenum (P = 0.04). Total mitochondrial protein and mitochondrial complex V activity were reduced for WB in the muscle (P = 0.0006 and P = 0.004), pancreas (P = 0.01 and P = 0.002), and duodenum (P = 0.001 and P = 0.002), respectively. Broilers with WB had increased malondialdehyde only in the pancreas (P = 0.01). Reduced GSH/GSSG ratios in the muscle (P = 0.01), pancreas (P < 0.05), and duodenum (P = 0.01) of WB broilers indicate more reactive oxygen species (ROS) in the tissues. *In vitro*, cells treated with an RNR inhibitor had reduced expression of RRM2 (P < 0.0001), Cytb (P = 0.0006), and UCP3 (P = 0.002) as well as reduced mitochondrial complex V activity (P = 0.001) and total mitochondrial proteins

(P = 0.01), indicating that RNR may regulate mitochondrial functions. In conclusion, reduced RRM2 expression may potentially reduce RNR function in the muscle, duodenum, and pancreas of broilers with WB and alter tissue function by increasing ROS production, mitochondrial abnormalities, and oxidative damage while reducing energy production.

KEYWORDS

gut health, meat quality, mitochondria, ribonucleotide reductase enzyme, woody breast

1 Introduction

Woody breast (WB) refers to a muscle condition resulting in broiler breast meat with abnormal texture and inferior fresh meat quality. Although WB meat is not a health risk to consumers, it has caused significant negative economic impacts on the poultry industry (Barbut, 2020). Although the specific causes of WB are unknown, fast growth rates and genetic selection of broilers have been suggested as potential factors (Kuttappan et al., 2016; Che et al., 2022). Several studies showed that mitochondrial abnormalities potentially play an important role in WB (Hisasaga, 2021; Shakeri et al., 2024) by increasing reactive oxygen species (ROS) in tissues, leading to severe oxidative stress, which in turn causes muscle damage and decreased ATP production (Wang et al., 2023). To date, there is no definitive solution to the WB problem. A few available findings suggest that dietary additives such as minerals may reduce the incidence of severe WB (Cauble et al., 2020; Kuttappan et al., 2021). Therefore, a healthier gut is hypothesized to potentially play a role in reducing WB incidence (Jia et al., 2022). Broilers with a healthier gut may absorb more nutrients and antioxidants (Obianwuna et al., 2023), which can help minimize oxidative stress. Among the three intestinal sections, the duodenum plays a crucial role in gut health as it is the primary site where food is mixed with digestive enzymes from the pancreas, initiating the process of nutrient absorption. The duodenum absorbs most of the minerals and vitamins (Basile et al., 2023), and the pancreas secretes digestive enzymes into the duodenum (Ravindran and Abdollahi, 2021). Therefore, alterations in the duodenum and pancreas could have a significant effect on mineral and vitamin absorption (Jia et al., 2022), which could possibly influence WB development in broilers.

Ribonucleotide reductase (RNR) is an enzyme involved in inflammatory pathways (Jones and Kounatidis, 2017) and mitochondrial function (Bourdon et al., 2007) and has been hypothesized to play a role in WB (Shakeri et al., 2023; Shakeri et al., 2024). RNR, subunit RRM2, is essential for the synthesis and replication of DNA and mitochondrial DNA (mtDNA) (Bourdon et al., 2007). Alterations in the expression of RRM2 are associated with higher ROS production, lower mtDNA content, and decreased levels of mitochondrial proteins, which impair ATP production in tissues (Shakeri et al., 2022). Previous studies *in vitro* have shown that RNR is essential for DNA synthesis and mtDNA function (Shadel, 2008; Salguero et al., 2012), while inhibition of RNR activity, such as with hydroxyurea, can lead to DNA replication arrest by depleting dNTP pools (Koç et al., 2004). However, there are no available data to show the effects of an RNR inhibitor such as hydroxyurea on the muscle cells of broiler chickens, and also how alterations in RNR activity may impact the mitochondrial function of muscle cells of broiler chickens. Therefore, testing the RNR inhibitor *in vitro* should be the first step before testing it *in vivo*. Our previous reports demonstrated that WB meat exhibited reduced RRM2 expression and increased mitochondrial abnormalities and oxidative stress (Shakeri et al., 2023; Shakeri et al., 2024). There is currently no available research examining the connection between RNR activity, gut health, and the WB condition. Therefore, the aim of this study was to investigate the role of RRM2 in duodenum health and mitochondrial function in broilers exhibiting WB. Furthermore, we also treated embryo fibroblasts with an RNR inhibitor to investigate its effects at the cellular level vs. WB. The current data are the first part of the experiment, testing the effects of RNR inhibitors *in vitro* before testing them *in vivo*. Since there have been no prior studies on the effects of RNR activity on meat quality, future research must explore various doses of the inhibitor in live birds to gain a clearer understanding of RNR's impact.

2 Materials and methods

2.1 Animals and sampling

Sixty 8-week-old Ross male broilers were obtained from a local research farm (University of Georgia, Athens, GA). Broilers were weighed individually and euthanized by cervical dislocation according to an approved institutional animal use protocol from the University of Georgia (A2022 04-029-Y3-A1). From the 60 carcasses, 20 were selected (10 severe WB and 10 normal) based on postmortem visual and tactile evaluations of the breast muscles by two trained experts. Scoring was from 1 to 3 for woody breast and white striping, with 1 considered normal and 3 considered severe. Scoring was from 0 to 2 for hemorrhage, with 0 considered normal and 2 considered severe. Breast fillets were deboned and scored for WB, white striping, and petechial hemorrhages according to a previously published paper (Shakeri et al., 2024). Breast muscle (*Pectoralis major*), duodenum, and pancreas tissue samples were collected <1 h postmortem. The collected tissues were immediately

flash frozen in liquid nitrogen and stored at −80°C or fixed in tissue freezing medium for histological analysis.

2.2 Mitochondria extraction

Tissue samples (100 mg) were rinsed with PBS and homogenized in 10 mL of mitochondria isolation buffer (210 mM of sucrose, 2 mM of EGTA, 40 mM of NaCl, and 30 mM of HEPES, pH 7.4) using a tissue grind pestle. The homogenized samples were centrifuged for 10 min at 900×*g* and 4°C. The supernatant was collected and centrifuged at 10,000×*g* for 10 min at 4°C. The obtained pellet was resuspended in 500 µL of resuspension buffer (10 mM of Tris and 1 mM of EDTA, pH 7.4) and used for the analysis.

2.3 Biochemical assays

Commercial kits were used to measure mitochondrial complex V activity (My BioScience, Cat. No.: MBS9719098, San Diego, CA, USA), total protein assay (Bio-Rad, Cat. No.: #5000001, USA), TBARS (thiobarbituric acid reactive substances) or MDA (Cayman, Cat. No.: 10009055, Ann Arbor, MI, USA), and GSH/GSSG ratio (glutathione/glutathione) (Invitrogen, Cat. No.: EIAGSHC, Waltham, MA, USA) based on protocols provided by the manufacturers. Each assay was performed with duplicate runs for all samples.

For complex V activity, 100 mg of tissue was mixed with the provided reagents, homogenized on ice, and then centrifuged at 600×*g* for 5 min at 4°C. The obtained supernatant was centrifuged at 11,000×*g* for 10 min at 4°C. The obtained pellet was suspended in a provided reagent by the manufacturer for the assay. Enzymatic activity was prepared with the reagents provided, and absorbance was read at 660 nm.

For TBARS, 25 mg of tissue was homogenized in RIPA buffer and centrifuged at 6,000×*g* for 10 min at 4°C to obtain the supernatant. Both samples and standards were prepared using SDS solution and color reagents after boiling them for 1 h. The standards were prepared based on the manufacturer's protocol. All samples and standards were loaded onto a plate and read at 530 nm.

For GSH/GSSG, 50 mg of tissue was homogenized with 5%SSA (provided by the manufacturer) and centrifuged at 14,000×*g* for 10 min at 4°C. Both GSH and GSSG standards were prepared based on the protocol. All standards and samples were mixed with detection reagents, and absorbance was read at 405 nm.

Mitochondria extracted from tissues were used to measure mitochondrial complex V activity and total mitochondrial protein, whereas homogenized tissues (provided protocol by the manufacturer) were used to measure MDA and GSH/GSSG ratio.

2.4 Cell culture

An immortalized cell line (Kim et al., 2001) was used to assess the effects of the RNR inhibitor (hydroxyurea, Thermo Fisher Scientific) on cells. Hydroxyurea is a cell apoptosis inducer that inhibits DNA synthesis. Cells were cultured to confluence (~3–4 × 10^7 cells) in T75 flasks with growth medium [DMEM (Gibco 10566016, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Gibco A3160402), and 1% penicillin–streptomycin [Gibco 15140148]) at 37°C in a humidified incubator (5% carbon dioxide) before using them for the experiment. Triplicates of the control and treated cultures were prepared and collected for analysis. Briefly, cells at 95%–100% confluence were treated with hydroxyurea (15 mg/mL final concentration in GM) for 24 h. The dosage of hydroxyurea was determined based on findings from previous studies conducted on mice and humans (Kinney et al., 1999; Geetha, 2018). Cells were then washed with D-PBS (Gibco) and trypsinized (0.25% Trypsin EDTA, Millipore Sigma, Rockville, MD) to detach cells from the culture flask. Fresh GM was added to deactivate trypsin and resuspend cells. Cell suspensions were then washed with D-PBS by centrifugation at 1,000 rpm for 8 min, and the cell pellets were recovered for the assays.

2.5 Quantitative real-time PCR

Total RNA was extracted from frozen tissues using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) and an RNeasy Mini

TABLE 1 Forward and reverse primers for qRT-PCR amplification and their functions.

Target	Full name/ primer reference	Function
RRM2	Ribonucleotide reductase regulatory subunit M2	Reduces apoptosis, DNA damage, and mitochondrial dysfunction
	Primer reference: NM, XM_040668831.2	
Cytb	Cytochrome b	Mitochondrial protein, energy metabolism, electron transport
	Primer reference: (Shakeri et al., 2023)	
UCP3	Uncoupling protein 3	Mitochondrial function and proton carrier
	Primer reference: NCBI, AF433170.2	
18S	Housekeeping gene	Internal controls for gene expression
	Primer reference: (Shakeri et al., 2024)	

TABLE 2 Broiler body weight, woody breast, white striping, and petechial hemorrhage scores for normal (N) and woody breast (WB) meat.

	N[a]	WB	SEM	*P*-value
Body weight (g)	3,831	4,228	± 85.35	0.0002
Woody breast[b]	1.05	2.80	± 0.09	<0.0001
White striping	1.10	1.60	± 0.12	0.0006
Petechial hemorrhage	0.60	1.30	± 0.34	0.05

[a]n = 10 birds per group.
[b]Scoring was from 1 to 3 for woody breast and white striping, with 1 considered normal and 3 considered severe. Scoring was from 0 to 2 for hemorrhage, with 0 considered normal and 2 considered severe.

Kit (Qiagen, Germantown, MD, USA). Total DNA was extracted from frozen tissues using a commercial QIAamp genomic DNA kit (Qiagen). Quantitative real-time PCR was performed with SYBR Green to measure gene expression used in this study. RNA, random hexamers, dNTPs, and free water were mixed (master mix 1), and then the mixture was heated at 65°C for 5 min and placed on ice for 2 min. Subsequently, 10 μL of Master Mix 2 (1× RT buffer, 25 mM of $MgCl_2$, 0.1 of DTT, RNaseOUT, and SuperScript III) was added to Master Mix 1. The thermocycler program was as follows: 65°C for 5 min, 4°C for 2 min, 25°C for 10 min, 50°C for 50 min, 85°C for 5 min, and 37°C for 20

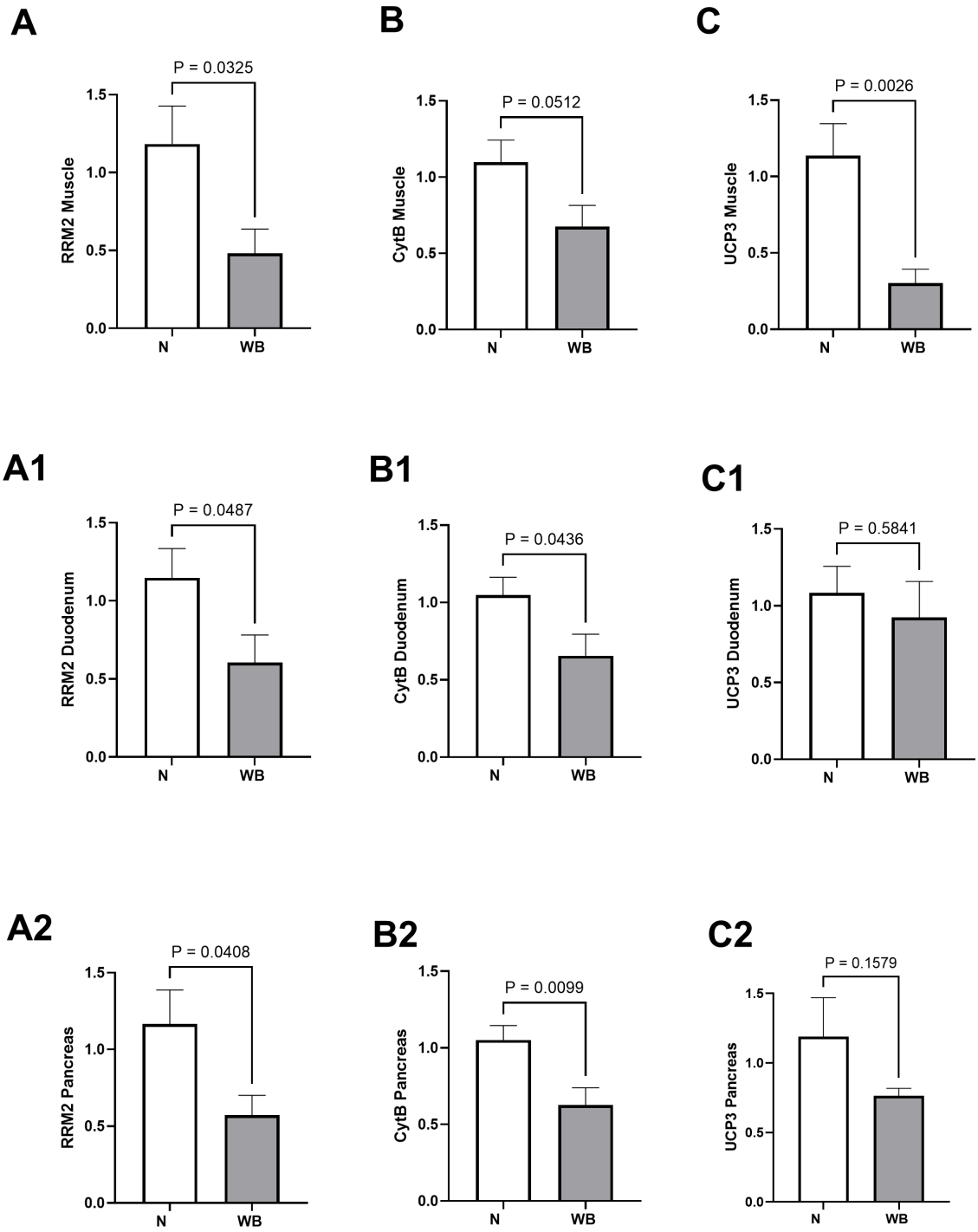

FIGURE 1
Expression (fold change) of genes related to mitochondrial function in the muscle, duodenum, and pancreas of normal (N) and woody breast (WB) broilers. In tissues, ribonucleotide reductase regulatory subunit M2 (RRM2, **A-A2**) and cytochrome b (Cytb, **B-B2**) were reduced in the muscle, duodenum, and pancreas, while uncoupling protein 3 (UCP3, **C-C2**) was only reduced in the muscle. Results were considered significant at $P < 0.05$ and trending toward significance at P = 0.05–0.1. Mean values are given as mean ± SE.

min. 18S was used to normalize the data, and fold changes were obtained using the $2^{-\Delta\Delta Ct}$ method. All genes used and their function are listed in Table 1.

2.6 Histology

Duodenal tissues were embedded in freezing medium (Triangle Biomedical Sciences, USA). The fixed tissues were cut (10 μm) using a cryostat and then stained with H&E using a standard protocol. All images were obtained (×10 magnification) using a light microscope (Olympus CX33, Japan). The number of lesions in N and WB was used to calculate the difference (Gurcan et al., 2009). One slide per sample was prepared for the analysis (a total of 20).

2.7 Statistical analysis

Data were analyzed using the *t*-test method (GraphPad Prism, version 10.2.2). *t*-test analysis was used to show differences between N vs. WB samples and between non-treated vs. treated cells with RNR inhibitor for 24 h. Results were considered significant at P <0.05 and trending toward significant at P = 0.05–0.1. Mean values are given as mean ± SE.

3 Results

3.1 Body weight and breast myopathy scores

Broiler weight and breast myopathy score data are presented in Table 2. WB broilers were heavier vs. N (N vs. WB, 3,831 vs. 4,228 g, P = 0.0002). Breast fillets from the WB group had greater white striping and petechial hemorrhage scores (1.60 vs. 1.10, P = 0.0006 and 1.30 vs. 0.60, P = 0.05, respectively) vs. N fillets.

3.2 Quantitative real-time PCR

Differences (N vs. WB) in the expression levels of RRM2, Cytb, and UCP3 are presented in the figures for muscle (Figures 1A–C),

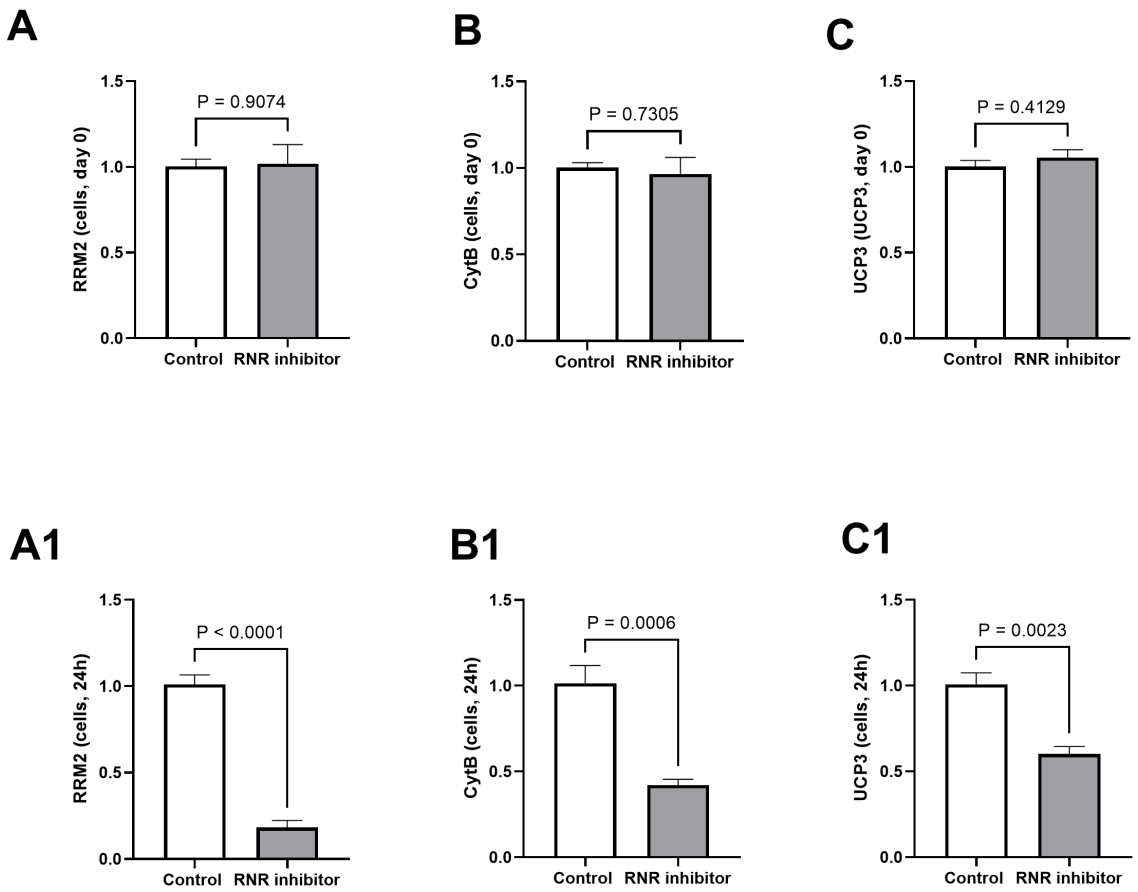

FIGURE 2
Expression (fold change) of genes related to mitochondrial function in cells before and after treating them with ribonucleotide reductase (RNR) inhibitor. In vitro, ribonucleotide reductase regulatory subunit M2 (RRM2, **A**, **A1**), cytochrome b (Cytb, **B**, **B1**), and uncoupling protein 3 (UCP3, **C**, **C1**) were reduced for cells treated with ribonucleotide reductase inhibitor for 24 h. Results were considered significant at P < 0.05 and trending toward significance at P = 0.05–0.1. Mean values are given as mean ± SE.

duodenum (Figures 1A1, B1, C1), and pancreas (Figures 1A2, B2, C2). RRM2 and Cytb expression levels were reduced for WB in the muscle (1.18 vs. 0.48, P = 0.03 and 1.09 vs. 0.67, P = 0.05), duodenum (1.14 vs. 0.60, P = 0.04 and 1.04 vs. 0.65, P = 0.04), and pancreas (1.16 vs. 0.57, P = 0.04 and 1.05 vs. 0.62, P = 0.009), respectively. UCP3 expression was reduced significantly for WB in the muscle (1.13 vs. 0.30, P = 0.002) and was reduced in the pancreas as well (1.19 vs. 0.76, P = 0.15) but not statistically significant. After 24 h, the cells treated with RNR inhibitor *in vitro* exhibited reduced expression of RRM2 (1.01 vs. 0.18, P < 0.0001), Cytb (1.01 vs. 0.42, P = 0.0006), and UCP3 (1.00 vs. 0.60, P = 0.002) (Figures 2A1, B1, C1) compared to non-treated cells. There were no significant differences between the cells before using the inhibitor (Figures 2A–C).

3.3 Biochemical assays

Data are presented in Table 3. Total mitochondrial protein and mitochondrial complex V activity were reduced for WB in the muscle (0.75 vs. 0.69 mg/mL, P = 0.0006 and 278.4 vs. 176.9 mM, P = 0.004), duodenum (0.24 vs. 0.14 mg/mL, P = 0.001 and 474.6 vs. 391.9 mM, P = 0.002), and pancreas (0.48 vs. 0.35 mg/mL, P = 0.01 and 446.8 vs. 441.6 mM, P = 0.002). GSH/GSSG ratio was reduced for WB in the muscle (783.4 vs. 550.7 µM, P = 0.01), duodenum (126.5 vs. 114.1 µM, P = 0.01), and pancreas (94.5 vs. 88.6 µM, P = 0.04). MDA increased for WB in the pancreas (4.94 vs. 5.12 nmol/mg, P = 0.01) and statistically insignificantly increased in the muscle and duodenum (5.22 vs. 5.48 nmol/mg, P = 0.18 and 4.58 vs. 5.39 nmol/mg, P = 0.07).

In vitro, cells treated with RNR inhibitor exhibited reduced total mitochondrial protein (1.96 vs. 1.69 mg/mL, P = 0.01) and reduced mitochondria complex V activity (651.4 vs. 603.2 mM, P = 0.001) compared to non-treated cells.

TABLE 3 Total mitochondrial protein, mitochondrial complex V activity, thiobarbituric acid reactive substances (TBARS), and glutathione/glutathione (GSH/GSSG) ratio in normal (N) and woody breast (WB) broilers or cells after 24 h treatment with RNR inhibitor.

	N	WB	SEM	*P*-value
Total mitochondrial protein (mg/mL)				
Muscle	0.75	0.69	± 0.01	0.0006
Duodenum	0.24	0.14	± 0.02	0.001
Pancreas	0.48	0.35	± 0.04	0.01
	Control	Inhibitor[a]		
Cells, day 0	1.36	1.29	± 0.08	0.46
Cells, 24 h	1.96	1.69	± 0.08	0.01
Mitochondrial complex V activity (mM)				
Muscle	278.4	176.9	± 30.83	0.004
Duodenum	474.6	391.9	± 23.43	0.002
Pancreas	446.8	441.6	± 1.50	0.002
	Control	Inhibitor		
Cells, day 0	604.1	617.5	± 23.93	0.60
Cells, 24 h	651.4	603.2	± 5.61	0.001
MDA or TBARS (nmol/mg)				
Muscle	5.22	5.48	± 0.18	0.18
Duodenum	4.58	5.39	± 0.29	0.01
Pancreas	4.94	5.12	± 0.17	0.07
GSH/GSSG (µM)				
Muscle	783.4	550.7	± 87.45	0.01
Duodenum	126.5	114.1	± 4.84	0.01
Pancreas	94.5	88.6	± 2.73	0.04

[a]Cells were treated with hydroxyurea for 24 h.

3.4 Histology

The number of moderate or severe lesions in duodenum images of WB was higher vs. N (1.1 vs. 1.7, P = 0.02) (Figure 3).

4 Discussion

RRM2 is essential for the synthesis and replication of DNA and mitochondrial DNA and is potentially involved in WB myopathy by controlling mitochondrial function and ROS production. Lowered expression of RRM2 in the muscle, duodenum, and pancreas leads to impaired mitochondrial function. Dysfunctional mitochondria lead to excessive production of ROS, resulting in increased oxidative damage to the tissues (Figure 4). The duodenum plays a major role in nutrient absorption, which is important for meat quality, while the pancreas produces and releases digestive enzymes into the duodenum to aid in digestion. Therefore, alterations in the duodenum and pancreas could have a significant effect on mineral and vitamin absorption, which possibly increases WB rate in broilers. Intestine health significantly impacts meat quality by impacting digestion, nutrient absorption, and overall animal health, which in turn affects the composition and characteristics of the meat produced (Chen et al., 2022).

In this study, we found that RRM2 expression was reduced in broilers with WB as well as in the cells treated with an RNR inhibitor. RRM2 expression reduction has been shown to impact ATP production pathways through altering DNA health and mitochondrial function (Mah et al., 2015; Brown et al., 2023) by increasing ROS and oxidative damage (Cho et al., 2015; Shakeri et al., 2022). The mitochondria are organelles where cells generate

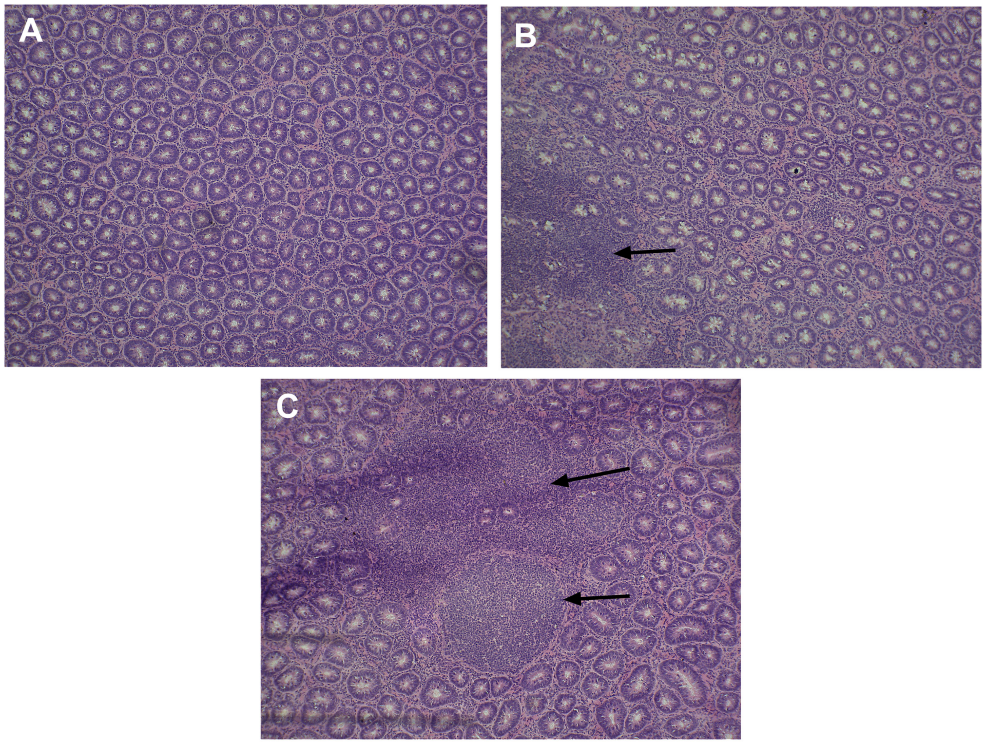

FIGURE 3
Differences between lesions (indicated by arrows) in the duodenum of normal **(A)** vs. two different grades of lesions in woody breast broilers **(B, C)** prepared by using hematoxylin and eosin stain. **(A)** Normal; **(B)** less infiltration; **(C)** moderate or severe infiltration.

ATP and reuse excessive ROS for other vital pathways; when the mitochondria do not function properly, ROS levels increase as ATP production decreases, leading to tissue damage (Wang et al., 2023; Shakeri et al., 2025). Furthermore, higher oxidative damage may increase lesions in tissues (Figure 3).

The expression levels of Cytb and UCP3 were reduced in the muscle, duodenum, and pancreas of broilers with WB and the cells treated with the RNR inhibitor, indicating a disruption in the normal function of the mitochondria. Cytb, a protein within the mitochondria, is encoded by mtDNA and plays a vital role in ATP production (Ahmad et al., 2018). UCP3 is a mitochondrial protein involved in energy metabolism and protecting against oxidative stress (Schrauwen, 2004) and is mainly expressed in skeletal muscle (Toime and Brand, 2010). The data also showed a reduction in mitochondrial complex V activity and total mitochondrial protein in broilers with WB and the cells treated with the RNR inhibitor. Mitochondrial complex V, also known as ATP synthase, is a crucial enzyme in the inner mitochondrial membrane that synthesizes ATP during oxidative phosphorylation (Jonckheere et al., 2012). Low total mitochondrial proteins often lead to mitochondrial disorders and impaired ATP production (Reverter et al., 2017).

Mitochondrial dysfunction is a primary driver of higher ROS in tissues (Bhatti et al., 2017). One of the best ways to determine ROS level is to measure the GSH/GSSG ratio. The ratio, representing the balance between oxidized glutathione, is a key indicator of cellular redox status and a marker of oxidative stress, with a lower ratio indicating increased ROS (Zitka et al., 2012). In fact, when ROS increases, GSH converts to GSSG to neutralize the ROS, leading to a decrease in the GSH/GSSG ratio (Zitka et al., 2012).

The current results showed that treating chicken muscle cells with the RNR inhibitor potentially increased mitochondrial dysfunction and oxidative damage, as observed in the WB. This supports our hypothesis that reducing RRM2 expression potentially increases ROS levels and oxidative damage in muscle tissue, which may contribute to WB occurrence. After confirming the inhibitor's effects (similar data as WB) *in vitro*, the next experiment will examine the hypothesis *in vivo* to observe the differences. Testing the inhibitor *in vitro* was necessary, as it had not been examined in broiler chicken cells before.

In conclusion, the reduction of RRM2 in WB broilers seemed to correlate with impaired mitochondrial function and increased ROS production, leading to increased oxidative damage to the duodenum and pancreas. Higher levels of oxidative damage can contribute to gut damage and impaired gut function, which can, in turn, disrupt normal absorption of nutrients of minerals or other essential nutrients, resulting in increased incidence and severity of the WB myopathy. Therefore, the study suggests that overexpressing RRM2 may reduce mitochondrial dysfunction and ROS production and consequently decrease WB occurrence.

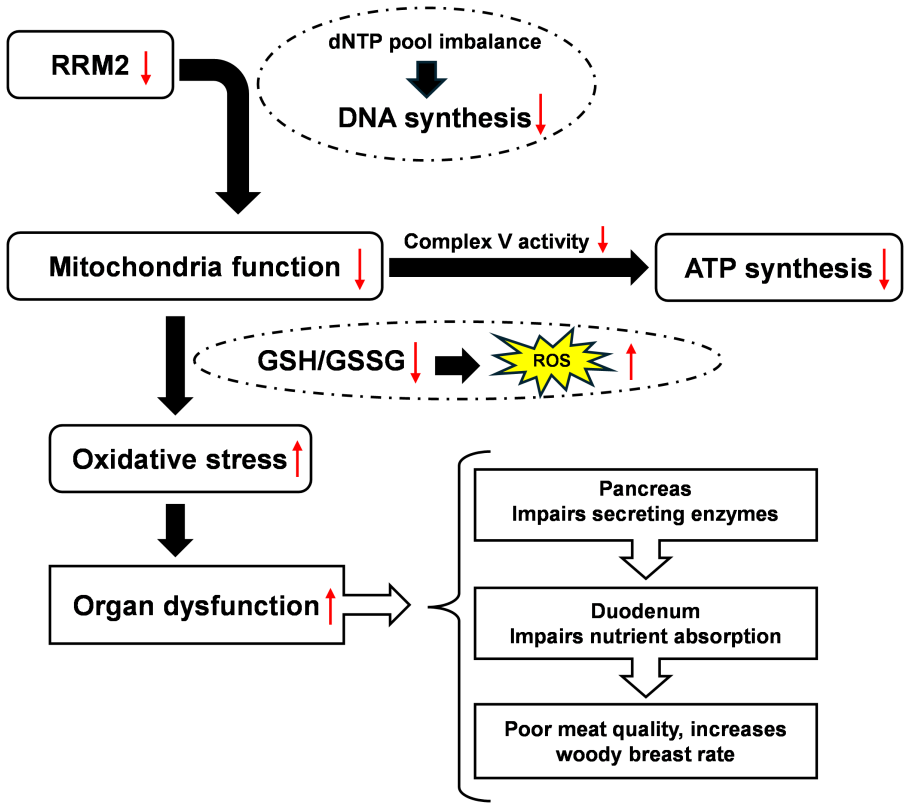

FIGURE 4
Reduction in the expression of ribonucleotide reductase, subunit RRM2, in the muscle, duodenum, and pancreas impairs mitochondrial function, which could potentially impact gut health and meat quality by increasing reactive oxygen species (ROS) production. Glutathione/glutathione (GSH/GSSG) ratio is an indicator of ROS level in tissues (the higher the ratio, the healthier the tissue).

Data availability statement

The data presented in the study are deposited in the Figshare repository, accession number 10.6084/m9.figshare.29646647. Further inquiries can be directed to the corresponding author(s).

Ethics statement

The animal study was conducted according to an approved institutional animal use protocol from the University of Georgia (A2022 04-029-Y3-A1). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MS: Methodology, Writing – review & editing, Software, Writing – original draft, Investigation, Validation, Formal analysis, Conceptualization, Data curation. EZ: Investigation, Writing – review & editing. CH: Investigation, Writing – review & editing. JC: Investigation, Writing – review & editing. HN: Writing – review & editing, Investigation. WK: Methodology, Writing – review & editing. BK: Writing – review & editing, Methodology. BB: Methodology, Funding acquisition, Writing – review & editing, Supervision, Validation, Resources.

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Conflict of interest

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