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Deciphering the underlying mechanisms of the oxidative perturbations and impaired meat quality in Wooden breast myopathy by label-free quantitative MS-based proteomics

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ABSTRACT

The study aimed to investigate biochemical mechanisms occurred in Wooden breast (WB) chicken meat, with attention to the impact on meat quality. Commercial chicken breasts were classified as Normal (N, n = 12), WB-M (moderate degree; focal hardness on cranial region, n = 12) and WB-S (severe degree; extreme and diffused hardness over the entire surface, n = 12). Samples were analyzed for physico-chemical properties, oxidative damage to lipids and proteins, and discriminating sarcoplasmic proteins by using a Q-Exactive mass spectrometer. WB meat presented impaired composition and functionality and higher levels of lipid and protein oxidation markers than N meat. The proteomic profile of WB-S presents a dynamic regulation of the relevant proteins involved in redox homeostasis, carbohydrate, protein and lipid metabolisms. Proteomics results demonstrate that the physiological and metabolic processes of muscles affected by WB myopathy are involved in combating the inflammatory process and in repairing the damaged tissue by oxidative stress.

1. Introduction

Wooden breast (WB) is a disorder that affects the breast muscle of rapidly growing birds. This disorder was provoked by the increased demand and consumption of chicken meat in the last decades, which pressured production systems to maximize meat production through intense genetic selection and improved efficiency of animal feed (Petracci et al. 2019). Excessive muscle hardness and histopathological alterations such as myofibrillar degeneration, lipidosis, fibrosis, and necrosis are inherent features of this myopathy (Sihvo, Immonen & Puolanne, 2014; Sihvo et al., 2017).

Muscles affected by WB myopathy exhibit deterioration in nutritional quality (i.e. protein, essential amino acids, unsaturated fatty acids) (Thanatsang et al. 2020; Liu et al., 2022), technological properties (i.e. water holding capacity) (Zhang et al., 2020), and sensorial properties (texture, appearance, color) (Dalle Zotte et al., 2017). Besides that, the acceptance and purchase intention of these meats by consumers is compromised (Rocha et al., 2022).

Oxidative stress seems to play a central role in the mechanisms behind WB pathology (Petracci et al., 2019). Previous studies focused on metabolomics and RNA-sequencing analyses, established metabolic pathways and some specific genes expression that potentially contribute to oxidative stress in WB muscle (Mutryn, et al., 2015; Abasht et al., 2016). Xing et al. (2021) observed altered levels of reactive oxygen species, oxidative products and antioxidant endogenous defenses in liver of animals affected, with all of these features being typical of mitochondrial dysfunction. On the other hand, Hasegawa et al. (2021) observed accumulation of lipofuscin in WB muscle, which is recognized as one of the indicators of oxidative stress. Several studies found increased lipid and protein markers (i.e. TBARS, protein carbonyls, free thiols) in breast meat of birds affected by WB disorder (Thanatsang et al., 2020; Hasegawa et al., 2021; Li et al., 2022). In addition, altered levels of antioxidant endogenous enzymes, such as catalase, superoxide dismutase, glutathione peroxidase, and glutathione S-transferase, are

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associated with imbalanced redox in WB meats (Xing et al., 2021). Proteomics has demonstrated to be an efficient tool to unveil physiologic mechanisms beyond breast myopathies such as white striping (Carvalho et al., 2020a). This technique allows the identification and level assessment of multiple protein, directly responsible for the phenotype of the muscle.

Given the remarkable significance of oxidative stress in chicken affected by WB myopathy, this study was designed to provide further insight into the biochemical and pathophysiological mechanisms involved in the onset and severity of the WB myopathy that drives the impairment of the chicken breast quality traits. To fulfil this objective, normal and WB meats were compared in terms quality traits, lipid and protein oxidation markers and proteome of sarcoplasmic proteins using a label-free mass spectromic-based proteomics approach. A better comprehension of the causes of the redox impairment in WB meats would enable the application of targeted prevention and alleviation strategies.

2. Material and methods

2.1. Samples, identification and classification

Thirty-six chicken breasts were collected from a commercial poultry slaughterhouse located in Paraíba, Brazil. The breasts used in the research were from birds slaughtered according to the "Regulation of Industrial and Sanitary Inspection of Products of Animal Origin (RIISPOA)" and "Technical Regulation of Technological and Hygienic-Sanitary Inspection of Poultry Meat" of the National Standards of Brazil. More specifically, the birds (Cobb genetic line), were stunned by electronarcosis prior to slaughter at 42–44 days of age. Samples were allocated to one of the following three groups based on the criteria described by Carvalho et al. (2020b): Normal ([N] *Pectoralis major* muscle without hardened areas on muscle surface), WB-moderate ([WB-M] *P. major* muscle with focal hardness on cranial region with/without petechiae), and WS-severe ([WB-S] *P. major* muscle with extreme and diffused hardness with petechiae). Twelve chicken breasts (n = 12) for each abovementioned group were collected and used for the present study.

2.2. Weight and muscle morphometry

After removing the skin, cartilage and bones that remained after deboning the chicken carcass, the breasts were weighed on a semi analytical balance (CE 2000, Marte Científica, Santa Rita do Sapucaí, Minas Gerais, Brazil) and subjected to geometrical evaluation with a digital caliper (684132, Lee Tools, Santo André, São Paulo, Brazil). Morphometric measurements of *P. major* muscle were determined according to Xing et al. (2020). Length was measured in the longest dimension of the muscle, whereas width was measured from the longest distance from side-to-side in the middle of the muscle. Top height was measured at the highest point in the cranial part, middle height was measured at the midpoint of the breast muscle length, and the bottom height was measured as the vertical distance from the caudal area by 25 mm in the dorsal direction.

2.3. Physical properties

pH was measured 24 h *post-mortem* by direct electrode insertion on the cranial area using a contact pH meter system (Testo 205, Testo do Brasil Instrumentos de Medição Ltda, Campinas, São Paulo, Brazil), according to the methodology proposed by Boulianne & King (1998). Color was measured using a Minolta colorimeter (Chroma Meter CR-400, Minolta Co., Osaka, Japan) in the CIELAB system (L*=lightness; a*=redness, and b*=yellowness) according to Carvalho et al. (2020a). The a*/b* ratio was used to establish the proportion between oxymyoglobin (MbO2) and metmyoglobin (MMb) (Olivo et al, 2001). Cooking loss (CL) was determined as described by Carvalho et al. (2017). Briefly, 5 g of breast meat were weighed, then the meat was cooked in a boiling water bath (100 °C) for 30 min and after cooking it was weighed again. Hardness was performed as described by Carvalho et al. (2020a) using a texturometer (TA.TXplus texturometer, Stable Micro Systems, Godalming, Surrey, UK). Raw samples were cut to size 25 × 25 × 10 mm (length × width × thickness, respectively). The samples were compressed twice to 50% of their original height with a cylindrical probe (5.08 cm diameter) at a test speed of 50 mm min^{-1}.

2.4. Chemical composition

Protein, moisture, ash and collagen contents were analyzed in Normal, WB-M and WB-S meats, according to the Association of Official Analytical Chemists (AOAC, 2000) described in items n° 928.08, 950.46, 920.153 and 920.26, respectively. The fat content was determined according to the methodology described by Folch et al. (1957).

2.5. Oxidative damage

2.5.1. Tbars

Lipid oxidation was determined according to the thiobarbituric acid-reactive substances (TBARS) method described by Ganhão et al. (2011). TBARS were measured at 532 nm using a spectrophotometer (Shimadzu UV-1800, Japan). A blank containing 2 mL of 3.86% perchloric acid and 2 mL of TBA reagent was used. The results from the samples were plotted against a standard curve prepared with concentrations of tetraethoxypropane (TEP) that ranged from 0.0583 to 1.1650 μg TEP mL^{-1}. The results were expressed as mg malondialdehyde (MDA) kg^{-1} breast meat. TBARS measurement was carried out in the raw samples and in the cooked samples after the determination of cooking loss.

2.5.2. Protein carbonyls

Protein oxidation was measured by the reaction of carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH) as described by Ganhão et al. (2010), with minor modifications. Protein concentration was calculated from the absorption at 280 nm using BSA as standard (0.06 to 3 mg/mL). The amount of carbonyls was expressed as nmol of carbonyl per mg of protein (nmol carbonyls mg $protein^{-1}$) using an absorption coefficient of 21.0 nM^{-1} cm^{-1} at 370 nm for protein hydrazones. Total carbonyl content was carried out in the raw samples and in the cooked samples after the determination of cooking loss.

2.5.3. Free thiols and disulphide bonds

Free thiols were analyzed following the methodology of Rysman et al. (2014). The thiol concentration was calculated based on a standard five points curve ranging from 2.5 to 500 μM cysteine in 6 M GuHCl in 1 M citric acid buffer (pH 4.5). Total thiols were determined with 4,4′-dithiodipyridine (4-DPS) as reducing agent. The disulphide content was calculated as the difference between total and free thiols divided by two.

2.6. Proteomic approach

Protein extraction. For the proteomic analysis, only N and WB-S were processed. Sarcoplasmic proteins fractions were extracted according to Zhu et al. (2011) with some modification. A total of 5 breasts from different animals for each condition were used. About 20 g of samples muscle was mixed with 80 mL of buffer containing 10 mM sodium phosphate, 0.1 N NaCl, 2 mM $MgCl_2$, 1 mM EGTA, pH 7.0, at 1200 g for 30 s. The homogenate was centrifuged at 600 g for 15 min at 4 °C, and the supernatant (non-pelleted solution) was decanted and saved. The pellet was resuspended in 80 mL of the same buffer, homogenized in vortex and centrifuged at the same setting. The supernatants were combined after centrifugation to obtain the final extract of sarcoplasmic proteins (SP). Protein content was determined in diluted SP extract (50 μL SP extract to 950 μL buffer) using Bradford method.

Label-free quantitative proteomic analyses. The preparation of chicken samples for proteomic analyses was carried out as follows. Briefly, 50 μg of from the sarcoplasmic fraction were partially run in SDS-PAGE to be picked up and undergone to dithiothreitol reduction and iodoacetamide-mediated alkylation. Subsequently, sequencing-grade trypsin (Promega, USA) and ProteaseMAX surfactant (Promega) were added to the gel pieces and incubated for 1 h at 50 °C, following manufacturer's instructions. A Q-Exactive Plus mass spectrometer coupled to a Dionex Ultimate 3000 RSLCnano (Thermo Scientific) analyzed around 0.75 μg from each digest. The gradient used ranged from 8 to 30% B (A: 0.1% formic acid (FA), B: acetonitrile, 0.1% FA) for 4 h on an Acclaim PepMap RSLC C18, 2 μm, 100 Å, 75 μm i.d. × 50 cm, nanoViper column (Thermo Scientific), thermostated at 45 °C in the oven compartment. Data were collected using a Top15 method for MS/MS scans (Delgado et al., 2019, 2017; Dolan et al., 2014). Comparative proteome abundance and data analysis was carried out by using MaxQuant software (Version 1.6.15.0; www. maxquant.org/downloads.htm), and Perseus (Version 1.6.14.0) to organize the data and perform statistical analysis. Carbamidomethylation of cysteines was set as a fixed modification; oxidation of methionines and acetylation of *N*-terminals were set as variable modifications. Database searching was performed against *Gallus gallus* protein database (downloaded December 2018, https://www.uniprot.org). The maximum peptide/protein false discovery rates (FDR) were set at 1% based on comparison to a reverse database. The LFQ algorithm was used to generate normalized spectral intensities and infer relative protein abundance. Proteins were identified with at least two peptides, and those proteins that matched to a contaminant database or the reverse database were removed, and proteins were only retained in final analysis if detected in at least three replicates from at least one condition. Quantitative analysis was performed using a *t*-test to compare treatments to the control. All proteins with $p < 0.05$ were included in the quantitative results. The qualitative analysis was also performed to detect proteins that were found in at least three replicates of a given WS level group but undetectable in the comparison group. The enrichment analyses were carried out using ClueGO software (v. 2.5.6). The Kappa score was set at 0.4. value and the *p*-value was analysed by Bonferroni step down and stablished at $p \leq 0.05$.

2.7. Statistical analysis

Data from physical-chemical composition and oxidative damage were analyzed by ANOVA. The means were compared using Tukey's test, using the different breast (Normal, WB-M, WB-S) as main factor. Statistical analyses were performed using XLSTAT software (version 2014.5.03, Addinsoft, New York, USA).

3. Results and discussion

3.1. Morphometry and physico-chemical properties

The results from the morphometric and physico-chemical analyses of chicken breasts are shown in Table 1. The occurrence of WB myopathy influenced weight and all morphometric parameters, mainly in breasts with severe degree, except the width ($p = 0.0628$), The mean weight was significantly higher ($p < 0.0001$) in WB samples (WB-S: 301.19 g, WB-M: 258.19 g) compared to Normal meat (232.25 g). The greatest length and bottom height (caudal area) of the breast was only observed in WB-S compared to Control (means 18.49 and 17.08 cm, respectively). In addition, the increase in the top (17 and 24% for WB-M and WS-S, respectively) and middle (27 and 33% for WB-M and WS-S, respectively) height was observed in both degrees of WB, compared to N. Similar results for weight and morphometric parameters were observed by other authors (Xing et al., 2020; Geronimo, Prudencio & Soares, 2022). Increased *Pectoralis major* muscle weight is result of genetic selection for fast growth and high yields (Petracci et al., 2015). The morphometric changes observed in WB meat can be attributed to the increase and growth of muscle cells. WB myopathy is characterized by histopathological features, including hypertrophy (Hosatani et al., 2020). According to Sihvo et al. (2014) and Sihvo et al. (2017), WB presents greater discrepancy in the muscle fibers, such as variability in shape, size and diameter.

Table 1
Morphometric and physico-chemical properties of chicken breast affected by the WB myopathy in the moderate and severe degrees.

Parameter	N	WB-M	WB-S	P-value
Weight[1]	232.25 ± 25.70^{c}	258.19 ± 14.53^{b}	301.01 ± 19.68^{a}	<0.0001
Length[2]	17.49 ± 0.83^{b}	17.45 ± 0.93^{b}	18.49 ± 0.58^{a}	0.0098
Width[2]	7.76 ± 0.48	7.72 ± 0.28	7.20 ± 0.80	0.0628
Top height[2]	28.97 ± 4.69^{b}	33.89 ± 3.04^{a}	35.98 ± 2.66^{a}	0.0007
Middle height[2]	22.40 ± 3.45^{b}	28.39 ± 3.04^{a}	29.68 ± 5.12^{a}	000,012
Bottom height[2]	12.28 ± 2.54^{b}	12.42 ± 1.65^{b}	17.08 ± 1.38^{a}	<0.0001
pH	5.78 ± 0.15	5.86 ± 0.16	5.86 ± 0.11	0.3578
L*	58.75 ± 2.72^{b}	59.35 ± 1.98^{b}	62.04 ± 1.69^{a}	0.0018
a*	1.01 ± 0.18^{b}	1.18 ± 0.30^{ab}	1.43 ± 0.32^{a}	0.0305
b*	2.98 ± 0.70^{c}	4.54 ± 1.00^{b}	6.83 ± 0.77^{a}	<0.0001
a*/b*	0.35 ± 0.02^{a}	0.27 ± 0.04^{b}	0.21 ± 0.02^{c}	<0.0001
Cooking Loss[3]	30.09 ± 4.72^{b}	34.26 ± 2.95^{ab}	36.74 ± 4.45^{a}	0.0044
Hardness[4]	35.99 ± 8.18^{b}	67.74 ± 15.57^{a}	74.70 ± 20.67^{a}	<0.0001
Moisture[5]	75.56 ± 0.78^{b}	76.16 ± 0.73^{ab}	76.58 ± 0.81^{a}	0.0128
Protein[5]	21.75 ± 0.89^{a}	20.50 ± 0.88^{b}	19.51 ± 1.11^{b}	<0.0001
Lipid[5]	2.24 ± 0.26^{b}	2.85 ± 0.64^{a}	3.24 ± 0.45^{a}	<0.0001
Ash[5]	1.00 ± 0.06^{b}	1.02 ± 0.09^{ab}	1.10 ± 0.09^{a}	0.0340
Collagen[5]	0.40 ± 0.06^{b}	0.48 ± 0.04^{a}	0.48 ± 0.04^{a}	0.0018

Lowercase letters on the same line differ significantly by Tukey's test ($p < 0.05$).
[1] Results expressed as g.
[2] Results expressed as cm.
[3] Results expressed as percentage.
[4] Results expressed as Newton.
[5] Results expressed as g 100 g^{-1}.

Physical properties (Fig. 1) were influenced by the presence of the myopathy, except pH ($p = 0.3578$). Higher L* was observed in the severe grade of WB myopathy ($p = 0.0018$) compared to WB-M and N. The a* parameter was higher in WB-S ($p = 0.0305$), while the value in WB-M (1.18) did not differ significantly from WB-S (1.43) and N (1.01) meats. Furthermore, WB-S showed the highest b* (6.83), followed by WB-M (4.54) and N (2.98) ($p < 0.0001$). That is, the more severe the degree of myopathy, the more yellowish the color of the meat. The a*/b* ratio was lower in meat with myopathies, showing values 22.9% and 40% lower for WB-M and WB-S, respectively, compared to N. Cooking loss (CL) was significantly higher in WB-S compared to N, while WB-S showed intermediate levels. An increase of 67.7% and 107.6% was observed in the hardness of WB-M (67.74 Newtons) and WB-S (74.70 Newtons) respectively, compared to the hardness of the N meat (35.99 Newtons). Regarding chemical composition, WB meat differed from N. The protein content was significantly lower ($p < 0.05$) in WB-M and WB-S (20.50 and 19.51 g/100 g, respectively) compared to N (21.75 g/100 g). The opposite effect was observed in relation to the lipid content, since WB-S and WB-M meats had a higher lipid content compared to N (3.24, 2.85 and 2.24 g/100 g, respectively). Collagen content was 20 % higher in chicken meat with myopathy (0.48 g/100 g) than the value observed in N (0.40 g/100 g). In addition, WB-S showed significantly higher values of ash ($p = 0.0340$) and moisture ($p = 0.0128$) contents in relation to N, while WB-M showed intermediate values.

Another well-known feature of meat affected by WB is excessive stiffness as a result of severe fibrosis (Sihvo et al., 2014; Sihvo et al., 2017; Hosatani et al., 2020). This may be explained by increased collagen content on the damaged tissue of WB meats compared to N (Table 1). According to Xing et al. (2020), the increased distributing of

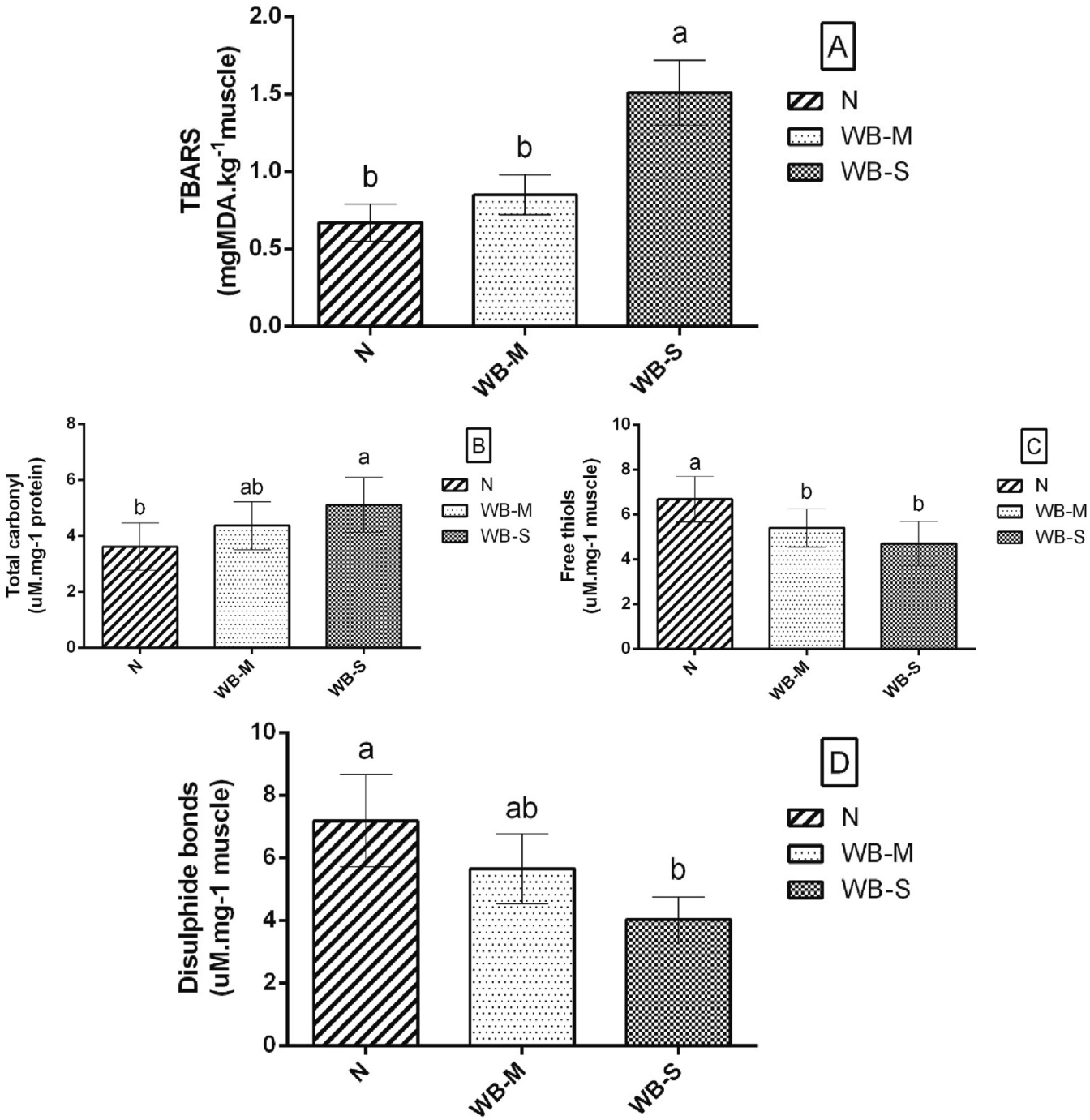

Fig. 1. Markers of oxidative damage in chicken breast affected by the WB myopathy in the moderate and severe degrees. Note: N: Normal breast meat; WB-M: Moderate degree of Wooden Breast myopathy; WB-S: Severe degree of Wooden Breast myopathy. A: Lipid oxidation marker; B-D: Protein oxidation markers. [a,b,c] Different lowercase letters differ significantly by Tukey's test ($p < 0.05$).

fibrotic proteins (collagen-I and fibrinectin) either in perimysium or endomysion is a pathological feature of WB meats. These authors observed a higher mRNA expression of collagen type I alpha 1 chain, collagen type III alpha 1 chain, fibronectin 1, α-smooth muscle actin, and connective tissue growth factor, in WB meats. Furthermore, Sihvo et al. (2014) highlighted that the collagenous structures resulting from fibrosis contributes to increasing the toughness of WB meat. In regards to the color displayed by breast meat, many researchers have reported that raw WB exhibited paler, reddish and yellowish color (Dalle Zotte et al. 2017; Zhuang & Bowker, 2018) than meat from unaffected birds. The most intense redness observed in WB meat may be due to the occurrence of petechiae (hemorrhagic lesion) on the muscle, especially in the more severe condition of this myopathy (Zhuang & Bowker, 2018). The presence of petechial hemorrhages in WB myopathy may be associated with circulatory failure due to muscle hypertrophy caused by the rapid growth rate of these birds (Clark & Velleman, 2016). Furthermore, the more yellowish coloration of WB meat may be related to more intense level of lipid oxidation. Xia et al., (2009) highlighted that the yellow pigments formation in meat are related to reactions between lipid oxidation products and the amine in proteins or in phospholipid head groups. Besides, the reduction in a*/b* ratio (Table 1) observed in affected meat by WB myopathy indicates increased discoloration of these meat, probably caused by myoglobin oxidation to metmyoglobin. According to Carvalho, Shimokomaki & Estévez (2017)

the change in poultry meat color is related to the impairment of redox homeostasis, leading to more intense levels of lipid and protein oxidation, thus promoting changes in pigments state of meat. In addition, WB meat presented poor cooking loss. Similar results were observed by Madruga et al. (2019) and Zhang et al. (2020). The greater susceptibility to protein oxidation (results discussed in the following section) observed in WB meats leads to severe protein modifications that may contribute to intense water loss during cooking. According to Bao and Ertbjerg (2019), changes in structure of muscle and their spatial arrangement resulting from protein oxidation may lead to protein cross-linking and affect protein net charges, influencing the muscle ability to retain water. Other factors that may be associated with greater CL is the reduced protein content and higher water content observed in WB meat (Table 1), both related to muscle degeneration. The increase in water content is linked to the fluid accumulation in muscle tissue due to inflammation process, while the decrease in protein content can be explained by the myofibrillar degeneration (Sihvo et al., 2014). According to Sihvo et al. (2017), the injured tissue due to myofibrillar degeneration is replaced by the deposition of fat and connective tissue, which explains the increase in lipid and collagen content, respectively, observed in this study (Table 1).

3.2. Oxidative damage

Results for the markers of oxidative damage measured in chicken breasts affected by WB myopathy are presented in Fig. 1. Breasts with a severe degree of myopathy had a significantly higher accretion of malondialdehyde and total protein carbonyls levels, which are products of lipid and protein oxidation, respectively (Estévez et al., 2019, 2021). However, in moderate degree of myopathy, it was not possible to observe a significant difference in these levels compared to N. The TBARS values (Fig. 1A) in WB-S (1.51 mg MDA kg^{-1} muscle) was 78% to 125% higher in compared to WS-M (0.85 mg MDA kg^{-1} muscle) and N (0.67 mg MDA kg^{-1} muscle), respectively. The total carbonyl content (Fig. 1B) was 41% higher in WB-S (5.11 μM mg-1 protein) compared to N (3.62 μM mg^{-1} protein). Lower levels of free thiols were observed in affected WB meats (4.70–5.40 μM thiols mg^{-1} muscle) compared to N (6.68 μM thiols mg^{-1} muscle) (Fig. 1C). In addition, there was less formation of disulfide bridges in the WB-S samples (Fig. 1D), with values being 44% lower compared to N (5.65 and 7.19 μM mg^{-1} muscle, respectively).

In general, the increased levels of lipid and protein oxidation in WB meat is directly related to the deterioration of its nutritional value (Thanatsang et al. 2020), formation of toxic products and off-flavor (Li et al., 2022), loss of color (Dalle Zotte, et al., 2017; Zhuang & Bowker, 2018), loss of essential amino acids (Thanatsang et al. 2020), decrease in the muscle's ability to retain water and negative impact on meat texture (Dalle Zotte, et al., 2017; Madruga et al., 2019; Zhang et al., 2020). In addition to compromising the quality and technological properties of WB meats, the highest level of TBARS and total carbonyl content and the lowest content of free thiols observed in this study is indicative of altered redox homeostasis, involving greater exposure of these meats to reactive oxygen species (ROS). Increased level of oxidation products in WB samples were also observed by Pan et al. (2021). Numerous factors can contribute to oxidative stress. Li et al. (2022) underline that high levels of lipid and protein oxidation may be caused by increased metabolic waste, poor blood and oxygen supply in the WB muscles. Furthermore, a potential genetic change can affect redox homeostasis by modifying the antioxidant activity of endogenous enzymes, thereby increasing ROS production in mitochondria (Lake & Abasht, 2020). Papah et al. (2018) observed up-regulation of genes fibromodulin (FMOD), activating transcription factor 3 (ATF3) and ankyrin repeat domain 1 (ANKRD1) related to stress/oxidative stress in WB. In addition, Pan et al. (2021) observed an increase in enzymes antioxidant activities and mitochondrial dysfunction in affected WB meats. Other factors that may explain the higher level of oxidative stress in WB meat could be related to lipidosis and the greater susceptibility to lipid peroxidation of fatty acids. According to Li et al. (2022), the change in lipid composition in affected WB meats is a result of intramuscular fat accumulation. Moreover, the higher content of monounsaturated and polyunsaturated fatty acids and lower content of saturated fatty acids were observed in breasts with WB myopathy (Gratta et al., 2023; Liu et al., 2022), with this modification making these meats more susceptible to lipid oxidation by ROS attack. Although the formation of disulfide bonds (DBs) is also an important marker of protein oxidation, WB meat had a reduced content compared to N meat. DBs are formed by the oxidation of two cysteine residues, and as a reduction in the content of free thiols in WB due to the oxidative protein process was observed, an increase in the formation of these crosslinks was expected. These results may be explained by two reasons: 1) The oxidation of thiols can form many products such as sulfenic, sulfinic and sulfonic acids and not just disulphide bonds (Schilter, 2017), and 2) The level of oxidation in the WB meats was so intense that the DBs could have been oxidized by singlet oxygen forming glutathionylated proteins (Jiang et al., 2021). In a previous study, we observed that most of the increased hardness in chicken breasts affected by breast myopathies was explained by the formation of crosslinking via Schiff base formation while disulphide bonds seemed to play a minor role (Da Rocha et al., 2020). Considering the role that sulphur-containing amino acids have on muscle redox status and meat quality, the depletion of these in chicken breasts affected by WB myopathy shows the sacrificial loss of these biomolecules as described in Estévez et al. (2020). This also suggests that dietary supplementation may be explored for trying to provide the affected muscle with antioxidant elements that seemed to be depleted/consumed in the pro-oxidative scenario caused during the onset of the myopathy.

3.3. Proteomics

Label-free comparative proteomic analyses were carried out for samples from every of the three WB conditions under study in this work. WB-S were compared to N in order to extract differentially abundant proteins (DAPs). Among these DAPs, one hundred and twenty-seven of them were found with differential relative abundance (fold change $\geq$ 1.20 or $\leq$ 0.82 and $p < 0.05$) in chicken breast muscles as affected by the occurrence of the myopathy (Table 2 and 3). Among these, 22 proteins were detected only in chicken breasts affected by severe WB myopathy, and 2 proteins only found in N breast meat. Furthermore, 67 proteins were observed in higher and 36 in lower abundance in WB-S. DAPs at higher amount in WB-S (Table 2) were mainly distributed by gene ontology enrichment in biological process (BP) related to 1) Response to oxidative stress, 2) Impaired myofibrillar protein homeostasis/function, 3) Hypoxia, inflammation and fibrosis, and 4) Apoptosis and necrosis. DEPs found in lower amount in WB-S (Fig. 1) were also distributed in BP related to 1) Failed energetic metabolism cell starvation, 2) Failed antioxidant response/depletion of antioxidant defenses, and 3) Apoptosis.

According to the results found, severe WB myopathy promotes changes in the proteome of chicken muscles. An increased concentration of proteins associated with lipid, protein and carbohydrate metabolism and endogenous antioxidant defense in the breast muscle of affected chickens were observed in this study.

Elevated protein concentration of Glutathione S-transferase (GSH-ST), Thioredoxin domain-containing protein (TXNDC), Glutathione peroxidase (GSH-PX) and peroxiredoxin-1 (PRDX1) was observed in affected birds. All of these proteins act to prevent or delay ROS-mediated oxidative damage to target tissues, playing an important role in maintaining redox homeostasis. GSH-ST enzymes have been previously related to greater susceptibility to oxidative stress-related diseases, including carcinogenesis (Strange, Spiteri, Ramachandran, & Fryer, 2001). According to Singhal et al. (2015), GSH-ST can reduce lipid hydroperoxides and may also detoxify numerous end-products of lipid peroxidation (LPO). TXNDC family proteins have a role in defense

Table 2
Proteins found in higher relative quantity in chicken breasts affected by severe WB myopathy.

Group	Protein	*p*-values	Log_2 Fold-change[1]	Biological function	Accession number
Response to oxidative stress	NTP_transferase domain-containing protein	–	S	GDP-mannose biosynthetic process	F1P574
	NmrA-like family domain-containing protein 1	–	S	Identical protein binding	A0A1D5PPI5
	Peptidase S1 domain-containing protein	–	S	Proteolysis	A0A1L1RUM8
	Non-specific serine/threonine protein kinase	–	S	Intracellular signal transduction, protein phosphorylation, negative regulation of potassium ion transmembrane transporter activity	E1C6W3; A0A3Q2U9U9
	Thioredoxin domain-containing protein	–	S	Protein folding	E1BSL7
	Glutathione S-transferase kappa	–	S	Glutathione metabolic process	F1N9G6
	Heat shock 27 kDa protein	0.0004	3.72	Protein refolding, negative regulation of oxidative stress-induced intrinsic apoptotic signaling pathway	F1P593; Q00649
	Chloride intracellular channel protein	0.0008	2.76	Chloride transport, negative regulation of ryanodine-sensitive calcium-release channel activity	Q5ZKI1
	Glutathione S-transferase	0.0329	2.53	Glutathione metabolic process, xenobiotic metabolic process	Q08392
	Ovotransferrin	0.0008	2.37	Iron ion homeostasis	E1BQC2
	Methanethiol oxidase	0.0101	2.12	Methanethiol oxidase activity, selenium binding	H9KYX6
	Thioredoxin domain-containing protein	0.0119	2.06	Peroxiredoxin activity, cell redox homeostasis	F1NNS8; A0A1D5PLH7
	GTP-binding protein	0.0013	2.03	Actin filament organization, stress fiber assembly	O93467; Q9PSX7
	Apolipoprotein A-I	0.0347	2.00	Cholesterol biosynthetic process, cholesterol homeostasis, lipid storage, lipoprotein biosynthetic/metabolic process, phospholipid homeostasis, positive regulation of stress fiber assembly, protein oxidation	P08250
	Endoplasmin	0.0028	1.93	Intracellular sequestering of iron ion, protein folding, ubiquitin-dependent ERAD pathway, negative regulation of apoptotic process, response to hypoxia	P08110; A0A1D5PPN9
	Protein disulfide-isomerase	0.0012	1.89	Protein disulfide isomerase activity, protein folding, response to endoplasmic reticulum stress	A0A1D5PV06; P09102; A0A1L1RZP5
	Glutathione transferase	0.0136	1.62	Glutathione metabolic process	E1BUB6
	Protein disulfide-isomerase A3	0.0019	1.62	Cellular response to interleukin-7, extrinsic apoptotic signaling pathway, protein folding, response to endoplasmic reticulum stress	Q8JG64
	DNA-(apurinic or apyrimidinic site) lyase	0.0369	1.57	Apoptotic process, DNA repair	F1NPA9
	Protein disulfide-isomerase A4	0.0095	1.57	Protein folding, response to endoplasmic reticulum stress	A0A1D5PWP7
	Aldo_ket_red domain-containing protein	0.0019	1.50	Alditol:NADP + 1-oxidoreductase activity	R4GG24
	Heat shock protein beta-8	0.0048	1.47	Cellular response to unfolded protein, positive regulation of aggrephagy	E1C6V0
	LRRNT domain-containing protein	0.0094	1.46	Extracellular matrix structural constituent	A0A1D5PAN0
	Sulfurtransferase	0.0190	1.35	Transsulfuration	A0A1D5PKN8
	Peptidyl-prolyl cis–trans isomerase	0.0392	1.35	Protein folding, protein peptidyl-prolyl isomerization	D0EKR3; A0A3Q3AT29
	10 kDa heat shock protein, mitochondrial	0.0279	1.33	Chaperone cofactor-dependent protein refolding	O42283; A0A1L1RZ94
	Glutathione peroxidase	0.0004	1.32	Response to oxidative stress	F1NYU4; A0A3Q3AIE0
	AdoHcyase_NAD domain-containing protein	0.0040	1.28	One-carbon metabolic process, S-adenosylmethionine cycle	A0A1D5PPA8; A0A1D5NWI2; A0A1D5PNB8
	Peroxiredoxin-1	0.0132	1.26	Cell redox homeostasis, leukocyte activation, removal of superoxide radicals	P0CB50
	S-formylglutathione hydrolase	0.0071	1.20	Formaldehyde catabolic process	E1BXC2
Impaired myofibrillar protein homeostasis/function	Cytochrome *c* oxidase polypeptide Va	–	S	Mitochondrial electron transport, cytochrome *c* to oxygen	E1C043
	AMP deaminase	–	S	AMP metabolic process, IMP biosynthetic process, IMP salvage	F1NG97
	Arp2/3 complex 34 kDa subunit	–	S	Actin filament polymerization, Arp2/3 complex-mediated actin nucleation, regulation of actin filament polymerization	F1P1K3

(*continued on next page*)

Table 2 (*continued*)

Group	Protein	*p*-values	Log_2 Fold-change[1]	Biological function	Accession number
	Macrophage-capping protein	–	S	Depolymerization, barbed-end actin filament capping, cell projection assembly	A0A1D5P7X3
	Myosin light polypeptide 6	–	S	Actomyosin structure organization, myofibril assembly	A0A3Q2U5H1; A0A1D5PEM4; A0A1L1RLN6; P02607
	Myosin-binding protein C, cardiac-type	–	S	Cell adhesion, positive regulation of ATPase activity, regulation of muscle filament sliding	A0A3Q2U3I8; A0A3Q3ACN5; F1NBZ9; Q90688; A0A3Q2UKP4; A0A1D5PKM9
	Ezrin	–	S	Actin filament bundle assembly, positive regulation of protein localization to early endosome, regulation of cell shape, regulation of organelle assembly	Q9YGW6
	UDP-glucose 6-dehydrogenase	0.0288	4.50	Carbohydrate metabolic process, glycosaminoglycan biosynthetic process, heparan sulfate proteoglycan biosynthetic process, protein hexamerixation	Q5F3T9
	Carbonic anhydrase	0.0088	3.42	One-carbon metabolic process	A0A1D5NTS2
	L-lactate dehydrogenase B chain	0.0011	3.36	Lactate metabolic process, pyruvate metabolic process	P00337
	Actin, cytoplasmic 2	0.0063	2.70	ATP binding	Q5ZMQ2
	Pyruvate kinase	0.0144	2.70	ATP binding	F1NW43
	ADF actin binding protein	0.0037	2.69	Actin filament polymerization	C7G537
	TRANSKETOLASE_1 domain-containing protein	0.0030	2.59	Regulation of growth, ribose phosphate biosynthetic process, glyceraldehyde-3-phosphate biosynthetic process	F1P1A5; A0A1D5NWL4
	Adenylyl cyclase-associated protein	0.0002	2.44	Actin cytoskeleton organization, cell morphogenesis	A0A1D5Q032
	Dystrophin	0.0083	2.28	Actin filament bundle assembly	A0A3Q2U000; A0A3Q2TWR1; A0A3Q2TUU4; A0A3Q2U719; F1NS97; A0A3Q3ABK2; A0A3Q2U0M3; A0A3Q2UJC3; A0A3Q2UPH9; A0A1D5PC47
	Creatine kinase B-type	0.0024	2.22	ATP biosynthetic process, phosphocreatine biosynthetic process	P05122
	Transaldolase	0.0055	2.18	Carbohydrate metabolic process	A0A1D5PC23
	Actin-depolymerizing factor	0.0217	2.14	Actin filament depolymerization, actin filament fragmentation, actin filament severing	Z4YJB8; P18359
	Gelsolin	0.0063	1.82	Actin filament severing	O93510
	Alpha-actinin-1	0.0188	1.76	Sarcomere organization, skeletal muscle fiber development, actin cytoskeleton organization, sarcomere organization	P05094; A0A1D5P9P3; A0A1D5PF13; A0A1D5P522
	Xin actin-binding repeat-containing protein 1	0.0003	1.69	Actin filament organization	Q91957; F1NUI2
	Amidohydro-rel domain-containing protein	0.0220	1.68	Actin crosslink formation, actin filament bundle assembly, response to axon injury	A0A1D5PZ51; A0A3Q2TWY6; F1NS48
	Actin-related protein 2/3 complex subunit 4	0.0031	1.68	Actin filament polymerization, Arp2/3 complex-mediated actin nucleation	F1P010; A0A3Q3ANM5
	Spectrin alpha chain, non-erythrocytic 1	0,0000	1.65	Actin filament capping, actin cytoskeleton organization	A0A1D5PVG1; A0A3Q2TVB7; A0A3Q2U3B7; A0A3Q2U5Z2; A0A3Q2U342; F1NHT3; A0A1D5PQC3; P07751
	Alpha-actinin-2	0,0226	1.52	Microspike assembly	P20111; A0A1D5PNV5; A0A1L1S0L2
	ATP synthase subunit beta	0,0055	1.45	Proton motive force-driven mitochondrial ATP synthesis	A0A1L1RY04
Hypoxia, Inflammation and fibrosis	CAP-Gly domain-containing linker protein 1	–	S	Cytoplasmic microtubule organization, positive regulation of microtubule polymerization	O42184
	Biliverdin reductase A	–	S	Heme catabolic process	A0A1D5NWT1
	Fibrillar collagen NC1 domain-containing protein	–	S	Cell adhesion, collagen fibril organization, extracellular matrix organization	A0A1D5PE74
	Collagen alpha-1(I) chain	–	S	Blood vessel development, cartilage development involved in endochondral bone morphogenesis, cellular response to amino acid stimulus, collagen biosynthetic process, collagen fibril organization	P02457; A0A1D5PYU1
	Ribonuclease homolog	–	S	Angiogenesis, defense response to Gram-negative bacterium, defense response to Gram-positive bacterium	P30374
	Procollagen-lysine 5-dioxygenase	–	S	Peptidyl-lysine hydroxylation	F1NXB0; A0A1D5P079; A0A1L1RSJ4
	Myoglobin	–	S	Oxygen transport, response to hypoxia	P02197
	EF hand-containing protein 1	–	S	Calcium ion binding	Q49B65
	Tenascin	0.0019	14.26	Cell adhesion, regulation of cell population proliferation	P10039; A0A1D5PJ88; A0A3Q2UL32; A0A1D5PZ80; A0A3Q2U5M0; A0A1D5NZY0
	Fibronectin	0.0000	13.82	Cell adhesion, cell migration, regulation of cell shape, acute-phase response, peptide cross-linking	A0A3Q2TW07; P11722; F1NJT4; A0A1D5NU50

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Table 2 (*continued*)

Group	Protein	*p*-values	Log_2 Fold-change[1]	Biological function	Accession number
	Desmin	0.0076	7.52	Intermediate filament organization, intermediate filament polymerization, skeletal muscle organ development	P02542
	Talin-1	0.0025	4.64	Cell adhesion, actin filament organization, endocytosis	P54939; E1C2S1; A0A1D5PTR5
	Vimentin	0.0048	3.60	Intermediate filament organization	F1NJ08; A0A1L1RXL9; P09654
	Protein S100-A6	0.0229	3.44	Cell cycle, positive regulation of cell division	Q98953
	Collagen alpha-3(VI) chain	0.0245	2.91	Cell adhesion	A0A3Q2UD12; F1P2F0; A0A1D5PGD5; A0A3Q3AR07; P15989; A0A3Q2U4U7
	Ferritin heavy chain	0.0019	2.77	Immune response, intracellular sequestering of iron ion, negative regulation of cell population proliferation	P08267
	Transforming growth factor-beta-induced protein ig-h3	0.0194	2.62	Cell adhesion, extracellular matrix organization	A0A1D5PXP9
	Fascin	0.0013	2.56	Actin filament bundle assembly, cell migration, establishment or maintenance of cell polarity	D5LPR1
	Tubulin alpha chain	0.0174	2.35	Microtubule cytoskeleton organization, mitotic cell cycle	A0A1D5P198; A0A1D5NW27; A0A1D5NXV1; P02552; A0A1L1RJP8; F1NWX0; P09644
	SERPIN domain-containing protein	0.0074	1.99	Negative regulation of angiogenesis, negative regulation of endopeptidase activity	E1C7H6
	Annexin A2	0.0007	1.98	Bone mineralization, calcium ion homeostasis, growth plate cartilage development	P17785
	Peptidyl-prolyl cis–trans isomerase B	0.0029	1.91	Negative regulation of collagen fibril organization, protein folding, protein peptidyl-prolyl isomerization	P24367; A0A1L1RRN4
	Alpha-1-acid glycoprotein	0.0431	1.86	Regulation of immune system process	Q8JIG5
	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase	0.0013	1.71	'de novo' IMP biosynthetic process	Q5XKY5; P31335
	Lumican	0.0448	1.63	Collagen fibril organization, visual perception	P51890
Apoptosis and necrosis	Acidic leucine-rich nuclear phosphoprotein 32 family member E	–	S	Histone exchange, regulation of apoptotic process	Q5F4A3; A0A3Q2UHZ1
	Parvalbumin, thymic	0.0086	4.58	Calcium ion binding	P19753
	Platelet-activating factor acetylhydrolase IB subunit alpha2	0.0020	2.11	Lipid catabolic process	Q5ZMS2; A0A1D5PMU2; A0A3Q2UC12
	Annexin A5	0.0002	1.81	Calcium ion homeostasis, negative regulation of coagulation	P17153
	Albumin	0.0219	1.71	Cellular response to starvation, negative regulation of apoptotic process, response to vitamin A	P19121
	Annexin	0.0303	1.68	Calcium ion binding	F1N9S7
	Cathepsin D	0.0067	1.34	Cell death, positive regulation of apoptotic process, proteolysis	Q05744

S: only found in WB-S chicken breast muscles.

against oxidative stress, and redox control in the plasma membrane, cytosol, endoplasmic reticulum, and nucleus (Cho et al., 2019). TXNDC are essential, among other functions, for reduction of protein disulphide and reduction of hydrogen peroxide (H_2O_2) (Hanschmann et al., 2013). GSH-PXs catalyzes the neutralization of ROS such as the decomposition of H_2O_2 or lipid peroxides by reduced glutathione (GSH) resulting in water or alcohol and oxidized glutathione (GSSG) (Sarıkaya and Doğan, 2020). Peroxiredoxins are cellular redox signaling, acting mainly in response to changes in production of hydrogen peroxide (Ledgerwood, Marshall and Weijman, 2017). The higher concentration of these proteins in WB-S meats is compatible with a pro-oxidative scenario such as that reported by WB muscles (Petracci et al., 2019). Yet, the attempt of the muscle to counteract the oxidation insults seemed to be ineffective looking at the higher levels of lipid (TBARS) and protein oxidation (total carbonyls) in breasts affected by the myopathy. Conversely, the fact that TXNDC is found in higher amount in the severe condition may explain the lower content of disulphide bonds observed in WB-S (Fig. 1D), as this enzyme is implicated in the reduction of disulphide bonds in proteins. Discriminating proteins corroborate that the oxidative stress is a crucial event behind the occurrence of this myopathy. The failed attempt by endogenous antioxidant defenses to maintain redox balance is in agreement with Xing et al. (2021) and Pan et al. (2020) who observed greater activity of antioxidant catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione S-transferase (GSH-ST) in chicken liver affected by WB myopathies.

Other proteins found in higher relative abundance in WB-S, such as Fibrillar collagen NC1 domain-containing protein, collagen alpha-1(I) chain, procollagen-lysine 5-dioxygenase, fibronectin, collagen alpha-3 (VI) chain, annexin A2, peptidyl-prolyl cis–trans isomerase B and Lumican are indicative of tissue damage and inflammatory process, since their biological function is related to the integrity and stability of collagen and tissue repair. The higher amount of these proteins could be related as an attempt of the muscle fiber to maintain its functionality in the face of fibrosis (Petracci et al., 2019). These proteome results are directly related to the higher collagen content observed in WB meat (Table 1).

The increased quantity of Arp2/3 complex 34 kDa subunit, Macrophage-capping protein, Ezrin, ADF actin binding protein, Adenylyl cyclase-associated protein, Dystrophin, Actin-depolymerizing factor, Gelsolin, Alpha-actinin-1, Xin actin-binding repeat-containing protein 1, Amidohydro-rel domain-containing protein, Actin-related protein 2/3 complex subunit 4, and Spectrin alpha chain, non-erythrocytic 1, are related to the actin structure organization, being directly related to the alteration of myofibrillar proteins function in WB

Table 3
Proteins found in lower relative quantity in chicken breasts affected by severe WB myopathy.

Group	Protein	p-values	Log_2 Fold-change[1]	Biological function	Accession number
Failed energetic metabolism_cell starvation	10-formyltetrahydrofolate dehydrogenase	–	N	10-formyltetrahydrofolate catabolic process, biosynthetic process, one-carbon metabolic process	F1P130
	Alpha-1,4 glucan phosphorylase	0.0033	0.33	Glycogen catabolic process	E1BSN7
	Alpha-1,4 glucan phosphorylase	0.0010	0.33	Carbohydrate metabolic process	A0A3Q3AC33
	Phosphorylase b kinase regulatory subunit	0.0070	0.36	Glycogen metabolic process	A0A1D5P061; A0A1D5PVZ4; E1BQZ7
	Creatine kinase S-type, mitochondrial	0.0018	0.46	Phosphocreatine biosynthetic process, phosphorylation	P11009; F1NAD3
	Glycerol-3-phosphate dehydrogenase 1	0.0030	0.47	Carbohydrate metabolic process, glycerol-3-phosphate metabolic process, NADH oxidation	A0A1D5PA73
	4-alpha-glucanotransferase	0.0031	0.48	Glycogen biosynthetic process, glycogen catabolic process	F1NX83; A0A1D5PF87
	Succinate–CoA ligase [ADP-forming] subunit beta, mitochondrial	0.0443	0.52	Succinyl-CoA metabolic process, tricarboxylic acid cycle	Q5F3B9
	Phosphoglycerate mutase	0.0004	0.53	Glycolytic process	Q5ZHV4
	Phosphoglycerate kinase	0.0001	0.53	Gluconeogenesis, glycolytic process, phosphorylation	F1NU17; P51903
	Succinate–CoA ligase [ADP/GDP-forming] subunit alpha, mitochondrial	0.0131	0.55	Tricarboxylic acid cycle	Q5ZL83
	Glyceraldehyde-3-phosphate dehydrogenase	0.0007	0.56	Gluconeogenesis, glycolytic process, negative regulation of apoptotic process	P00356
	2-phospho-D-glycerate hydro-lyase	0.0408	0.56	Glycolytic process	A0A1D5PSH6
	Guanidinoacetate *N*-methyltransferase	0.0008	0.59	Creatine biosynthetic process, methylation	A0A1D5P3Q3; A0A1L1RRC8; A0A3Q2UE67
	Gal_mutarotas_2 domain-containing protein	0.0056	0.60	Carbohydrate metabolic process, glycan processing	E1BTT7
	Adenylosuccinate lyase	0.0012	0.61	'de novo' AMP biosynthetic process, 'de novo' IMP biosynthetic process	P21265; E1BR00
	Beta-enolase	0.0016	0.61	Glycolytic process	P07322
	Phosphoglycerate mutase 1	0.0009	0.62	Gluconeogenesis, glycolytic process	Q5ZLN1
	Pyruvate kinase PKM	0.0039	0.62	Glycolytic process	P00548; A0A1D5P9V0
	Glucose-6-phosphate isomerase	0.0043	0.63	Gluconeogenesis, glucose 6-phosphate metabolic process, glycolytic process	A0A1L1RQ91; F1NIJ6; A0A1L1RPE8; A0A1D5PRS4
	Fumarate hydratase, mitochondrial	0.0045	0.64	Fumarate metabolic process, malate metabolic process, tricarboxylic acid cycle	Q5ZLD1
	L-lactate dehydrogenase	0.0040	0.65	Lactate metabolic process, pyruvate metabolic process, carboxylic acid metabolic process	E1BTT8; P00340; A0A1D5NZ01
	Triosephosphate isomerase	0.0204	0.66	Gluconeogenesis, glyceraldehyde-3-phosphate biosynthetic process, glycerol catabolic process, glycolytic process, methylglyoxal biosynthetic process	P00940
	Malic enzyme	0.0013	0.67	Malate metabolic process, pyruvate metabolic process	A0A3Q2UB37; F1P0Y6; A0A1D5P779
	Aspartate aminotransferase	0.0253	0.70	Biosynthetic process, cellular amino acid metabolic process, aspartate metabolic process	A0A1L1RPR4; F1P180; P00508
	SGNH_hydro domain-containing protein	0.0126	0.70	Lipid catabolic process	F1NKW4
	Branched-chain-amino-acid aminotransferase	0.0195	0.71	Cellular amino acid biosynthetic process, branched-chain amino acid biosynthetic process, leucine biosynthetic process, valine biosynthetic process	A0A3Q3AF25; A0A3Q2UDM7; E1BSF5; A0A3Q2U4M1
	FIST_C domain-containing protein	0.0300	0.71	Positive regulation of proteasomal ubiquitin-dependent protein catabolic process, protein polyubiquitination, regulation of skeletal muscle fiber development	Q5ZLD9
	Serine/threonine-protein phosphatase	0.0233	0.75	Cell division, glycogen metabolic process	A0A1D5P888; A0A1L1RMC6
	CBM21 domain-containing protein	0.0168	0.75	Regulation of glycogen biosynthetic process	A0A1L1S103; A0A3Q2UB77
Failed antiox response/ depletion of antiox defenses	PDZ domain-containing protein	–	N	Positive regulation of actin filament bundle assembly	F1NH40
	Aldedh domain-containing protein	0.0001	0.56	Aldehyde dehydrogenase (NAD +) activity	E1BT93
	Thioredoxin domain-containing protein 17	0.0216	0.70	Protein-disulfide reductase (NAD(P)) activity	R4GMD9
	Hemoglobin subunit alpha-A	0.0373	0.71	Cellular oxidant detoxification, hydrogen peroxide catabolic process	P01994
	Protein/nucleic acid deglycase DJ-1	0.0024	0.74	Autophagy, cellular response to hydrogen peroxide, cellular response to oxidative stress, DNA repair, hydrogen peroxide metabolic process, inflammatory response	Q8UW59; D5M8S2
	Peroxiredoxin-6	0.0478	0.75	Cell redox homeostasis, lipid catabolic process	F1NBV0; Q5ZJF4
	Dihydrolipoyl dehydrogenase	0.0345	0.82	Cell redox homeostasis	Q5ZM32
Apoptosis	Proteasome inhibitor PI31 subunit	0.0097	0.69	Ubiquitin-dependent protein catabolic process	Q5ZJL3

N: only found in normal chicken breast muscles.

abnormality. In addition, Arp2/3 complex, ezrin, alpha-actin and Xin actin-binding repeat-containing protein 1 are specifically involved in cross-linking actin filaments (May, 2001; Courson & Rock, 2010; Lees, 2015; Kawaguchi, et al. 2017), which contributes to the strengthening of actin filaments making these more mechanically resistant. Thus, their increased quantities may result in the higher hardness observed in WB meat (Table 1). In particular Gelsilin is one of the proteins responsible for assembly and disassembly of actin filaments, its functions include cell motility, proliferation and apoptosis. Furthermore, it plays an important role in localizing inflammation and preventing systemic escape of pro-inflammatory lipids (Gungor et al., 2016). Gelsilin increment may indicate an attempt to combat the inflammatory process resulting from WB myopathy. Infiltration of the muscle tissue and organs (liver) by inflammatory cells has been reported in WB myopathy (Sihvo et al., 2014; Velleman & Clark, 2015, Xing et al., 2021).

The higher abundance of cathepsin D and albumin reveals that WB chicken breasts are more susceptible to apoptosis. In general, Cathepsin D can either induce the programmed cell death in presence of cytotoxic factors, being activated by dysfunction of mitochondria (Minarowska et al., 2007). In addition, the higher amount of albumin could be interpreted as an attempt of the muscle fiber to react to mitochondrial damage induced by reactive oxygen species (Liu et al., 2012).

Lower relative abundance of key proteins in energy and carbohydrate metabolism such as creatine kinase (CK), pyruvate kinase (PK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) show the severe perturbation of energetic metabolism and inefficient mitochondrial function and may, in turn, lead to oxidative stress (Carvalho et al., 2020a,b). All of these enzymes are known to be indicators of WB muscle stress and cellular damage (Kawasaki, Yoshida & Watanabe, 2016; Thanatsang, et al. 2020; Carvalho et al., 2020a,b; Kong et al. 2021). Carvalho et al. (2020) observed lower relative abundance of PK, CK, and LDH in meats affected by white striping myopathy, which also originates from intense genetic selection. According to Thanatsang et al. (2020), the expression of LDH gene in WB muscle tissue was lower than the N meat. However, Kawasaki et al. (2016) and Kong et al. (2021) observed greater activity of CK and AST in serum or breast tissue of affected WB compared to N. Other group of DAPs such as Hemoglobin subunit alpha-A, DJ-1, Peroxiredoxin-6 and Dihydrolipoyl dehydrogenase are implicated in cellular response to oxidative stress. The minor abundance of these proteins confirms the failed attempt of the WB muscle to act against oxidative stress and/or the consumption of certain elements of this antioxidant response.

4. Conclusions

Current findings indicate that altered oxidative status is a common feature of WB myopathy in broilers. Oxidative stress involves accretion of malondialdehyde and total protein carbonyls and depletion of sulphur protein components, which seem to have a direct impact on technological characteristics, color and texture of the affected meats. The proteomic analyses suggest: 1) an alteration in redox homeostasis with higher abundance of proteins directly involved in the repair of oxidative damage in tissue, and 2) a failed attempt by the muscle to maintain or recover its functionality with increased amount of proteins related to maintaining muscle structure and combating the inflammatory process. The results from the present study indicates that antioxidant strategies to be applied *in vivo* may help the living muscle in counteracting the oxidative stress and the occurrence of its negative effects on meat quality. Looking at the results, supplementation with sulphur amino acids and other micronutrients with antioxidant potential in muscle is to be explored given the crucial role of oxidative stress in the onset of the myopathy and the impaired quality traits of the meat. Future studies should look into the underlying causes of the oxidative stress in chicken WB and address other means of alleviation such as using lower-growth genetic lines.

CRediT authorship contribution statement

Leila M. Carvalho: Data curation, Methodology, Formal analysis, Supervision, Validation, Writing – original draft. **Thayse C. Rocha:** Data curation, Methodology, Formal analysis, Writing – review & editing. **Josué Delgado:** Data curation, Methodology, Formal analysis, Validation, Writing – review & editing. **Silvia Díaz-Velasco:** Data curation, Methodology, Formal analysis, Writing – review & editing. **Marta S. Madruga:** Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing. **Mario Estévez:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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