UNIVERSIDADE FEDERAL DA PARAÍBA
CENTRO DE TECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA DE ALIMENTOS

LEILA MOREIRA DE CARVALHO

OCORRÊNCIA DE MIOPATIAS EMERGENTES E A QUALIDADE E ESTABILIDADE OXIDATIVA DE PEITOS DE FRANGO ESTRIADO (*WHITE STRIPING*)

JOÃO PESSOA
2020

LEILA MOREIRA DE CARVALHO

OCORRÊNCIA DE MIOPATIAS EMERGENTES E A QUALIDADE E ESTABILIDADE OXIDATIVA DE PEITOS DE FRANGO ESTRIADO (*WHITE STRIPING*)

Tese apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos da Universidade Federal da Paraíba em cumprimento aos requisitos para obtenção do título de Doutor em Ciência e Tecnologia de Alimentos.

Orientador: Prof. Dra. MARTA SUELY MADRUGA

Coorientador: Prof. Dr. MARIO ESTÉVEZ GARCÍA

JOÃO PESSOA

2020

Catalogação na publicação
Seção de Catalogação e Classificação

C331o Carvalho, Leila Moreira de.
Ocorrência de miopatias emergentes e a qualidade e estabilidade oxidativa de peitos de frango estriado (White Striping) / Leila Moreira de Carvalho. - João Pessoa, 2020.
175 f. : il.

Orientação: Marta Suely Madruga.
Tese (Doutorado) - UFPB/CT.

1. Wooden Breast. 2. White Striping. 3. NIRS. 4. Emoções. 5. Estresse oxidativo. I. Suely Madruga, Marta. II. Título.

UFPB/BC

LEILA MOREIRA DE CARVALHO

OCORRÊNCIA DE MIOPATIAS EMERGENTES E A QUALIDADE E ESTABILIDADE OXIDATIVA DE PEITOS DE FRANGO ESTRIADO (*WHITE STRIPING*)

Tese apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos da Universidade Federal da Paraíba para obtenção do título de Doutor em Ciência e Tecnologia de Alimentos.

Tese aprovada em 21/05/2020

BANCA EXAMINADORA

Dra. Marta Suely Madruga – Universidade Federal da Paraíba

Orientadora

Dr. Mario Estévez García – Universidade de Extremadura (Cáceres/Espanha)

Coorientador

Dra. Elza Iouko Ida – Universidade Estadual de Londrina

Examinador Externo

Dr. Douglas Fernandes Barbin – Universidade Estadual de Campinas

Examinador Externo

Dra. Mayka Reghiany Pedrão – Universidade Tecnológica Federal do Paraná

Examinador Externo

Dr. Fábio Anderson Pereira da Silva – Universidade Federal da Paraíba

Examinador Interno

AGRADECIMENTOS

À Universidade Federal da Paraíba, pelos seus recursos físicos, financeiros e humanos, e por proporcionar meu desenvolvimento científico desde a graduação.

À Universidade de Extremadura, por permitir o desenvolvimento do meu projeto de investigação e aperfeiçoamento científico em sua infraestrutura física.

Ao CNPq pelo financiamento do Projeto de Pesquisa no Brasil (430832/2016-8) e pelo suporte financeiro para realização do Doutorado Sanduíche no Exterior (208241/2017-5).

À CAPES pela concessão de bolsa e auxílio financeiro para a realização deste doutorado.

Aos coordenadores, secretários e a todos os docentes que compõem o Programa de Pós Graduação em Ciência e Tecnologia de Alimentos (PPGCTA), pelo suporte e por fornecer condições para a realização da pesquisa.

A minha querida orientadora, Profa. Dra. Marta Suely Madruga, por toda dedicação, ensinamentos e motivação ao longo de toda jornada. Serei eternamente grata pelas oportunidades e amizade ao longo destes seis anos de trabalho juntas.

Ao meu coorientador Prof. Dr. Mario Estévez García, por sua supervisão, orientação e disponibilidade. Serei eternamente grata pela experiência vivenciada no Doutorado Sanduíche.

A banca examinadora desta pesquisa, professores Dra. Elza Iouko Ida, Dra. Mayka Reghiany Pedrão e Dr. Fábio Anderson Pereira da Silva pelas brilhantes considerações que guiaram a confecção final deste trabalho, e também ao Prof. Dr. Douglas Fernandes Barbin cujas observações foram igualmente imprescindíveis e pelo seu suporte e apoio na aprendizagem de técnicas de análise multivariada.

Ao professor Dr. Massami Shimokomaki (*in memoriam*), por propor o tema desta tese.

A professora Dra. Sonia Ventanas por toda a ajuda e amizade durante a estância na Universidade de Extremadura.

As técnicas de laboratório Dra. Mércia de Sousa Galvão e Inmaculada Flores Cerro, e ao pesquisador Dr. Alberto González-Mohino Jiménez, pela amizade e apoio na realização das análises.

Aos “Madrugas”, que me ajudaram na realização das análises e por fazerem do nosso ambiente organizacional um local agradável, divertido, saudável e harmônico. Muito obrigada pela amizade e compreensão de todos!

A Deus, pela oportunidade, pelas bênçãos concedidas, por seu infinito e inesgotável amor, e por estar comigo nos momentos de certezas e incertezas. Meus mais sinceros agradecimentos!

Ao meu pai, Humberto Sérgio Pires de Carvalho pelo carinho, incentivo e esforço em garantir uma educação de qualidade, e a minha mãe Gillian Moreira de Carvalho, pelo amor, dedicação e por me suportar nos momentos de estresse. Tenho muito orgulho por ter sido agraciada por Deus em ter vocês como meus pais. Amo muito vocês!

Às minhas irmãs, Lillian Moreira de Carvalho e Jéssica Moreira de Carvalho pelo apoio, carinho e palavras de incentivo ao longo de toda a jornada.

Aos meus avós, Geraldo Moreira da Silva e Evandi Moreira da Silva pelo exemplo de honestidade, pelo amor e por me distrair nos momentos de maior tensão.

À Marisa Vallejo e Fernando Silvestre, que me deram todo o apoio emocional e espiritual enquanto estive em Cáceres, Espanha. Vocês fizeram com que a cidade Cáceres pudesse ser chamada de meu segundo lar. Hoje vocês não são considerados apenas meus amigos, os considero família.

A todos que, de forma direta ou indireta, tenham contribuído para a realização deste trabalho.

“*Quão melhor é adquirir a sabedoria do que o ouro! E quão mais excelente é adquirir a prudência do que a prata*” (Provérbios 16:16)

RESUMO

O aumento mundial da demanda e do consumo da carne de frango nas últimas décadas causou uma pressão nos sistemas de criação que buscaram otimizar a produção de carne de frango. Para cumprir o objetivo, a intensa seleção genética e o aprimoramento dos programas de alimentação animal resultaram animais com maior ganho de peso em um período de tempo reduzido, associado a um menor custo. Como consequência dessa pressão biológica surgiram novas miopatias, entre elas o peito estriado (*White striping* – WS) e peito amadeirado (*Wooden breast* – WB). O objetivo deste trabalho foi analisar a ocorrência das miopatias WS e WB no Nordeste do Brasil, avaliar o uso de espectroscopia do infravermelho próximo (NIRS) para classificação de peitos acometidos por estas miopatias em planta industrial de abate, investigar o efeito da miopatia WS na qualidade da carne, com foco na compreensão dos mecanismos moleculares por trás do estresse oxidativo e avaliar o quanto a aceitabilidade, intenção de compra e emoções evocadas nos consumidores foi afetada diante da conscientização das causas da miopatia WS. Em um primeiro estudo, foi verificado a ocorrência de peitos WS e WB (isoladas e/ou combinadas) em aves de diferentes faixas de idade de abate (4-5, 6-7, 8-9 e 65 semanas) em uma planta comercial usando método tradicional de detecção (palpação e aspecto visual do músculo). A espectroscopia de infravermelho próximo (NIRS) associado aos modelos de classificação SPA-LDA e SIMCA foi utilizada como ferramenta para distinguir peitos Normal (N), WS, WB e WS/WB. Em um segundo estudo, peitos de frango WS, com graus moderado e severo da miopatia, foram analisados quanto às propriedades físico-químicas, características de qualidade, defesas antioxidantes endógenas, danos oxidativos à fração lipídica e proteica, e discriminação das proteínas sarcoplasmáticas usando espectrômetro de massa Q-Exactive Orbitrap. Em um terceiro estudo, foi avaliada a atitude dos consumidores espanhóis quanto à conscientização das causas da miopatia WS, e como o reconhecimento desses peitos influenciam a aceitabilidade, intenção de compra e emoções evocadas pelos consumidores. Os resultados demostraram uma elevada ocorrência das miopatias WS, WB e WS/WB no Brasil, e que estas, foram mais frequentes e apresentaram-se de forma mais agravada em aves de maior idade de abate. Além disso, carnes com miopatias apresentaram um comprometimento do valor nutricional. A técnica NIRS associada ao modelo de classificação SPA-LDA apresentaram elevada acuracidade na identificação de carne Normal (sem miopatias), WS e WB (isolada e combinada a WS), independentemente da idade de abate das aves. Carnes afetadas por WS apresentaram maior depleção de tióis livres, e formação expressiva de malonaldeído, alisina e base de Schiff; além de comprometimento da atividade das enzimas antioxidantes endógenas catalase, superóxido dismutase e glutationa peroxidase. O estudo proteômico revelou uma tentativa fracassada de manter a função biológica da fibra muscular e a homeostase redox. Peitos de frango estriado (WS) apresentaram baixa aceitabilidade e intenção de compra, principalmente quando foi fornecida aos consumidores a informação sobre as causas e consequência desta miopatia. O processo de cozimento da carne foi capaz de mascarar a visualização das estrias brancas, levando os consumidores a ter preferência pela carne WS em relação a carne Normal quando em condições não informada de consumo; enquanto que em condições informadas, 20% dos consumidores tenderam a rejeitar o consumo da carne WS. Ademais, o perfil emocional dos consumidores foi significativamente afetado pela conscientização sobre as miopatias WS, pois os consumidores se sentiram mais “interessados” e “entusiasmados” e menos “bem-humorados”, “amorosos” e “amistosos” ao consumir essas carnes. A partir dos resultados gerados nessa pesquisa foi possível concluir que o nível de ocorrência e o grau das miopatias no músculo do peito está diretamente relacionada à idade de abate das aves. Também foi possível confirmar o uso promissor da técnica NIRS na identificação de miopatias em linhas de processamento industrial. Além disso, revelou que peitos WS apresentam a maior susceptibilidade ao estresse oxidativo, e consequente comprometimento dos processos fisiológicos e metabólicos do músculo. Além disso, mostrou que o benefício da informação sobre o que os consumidores ingerem comprometeu a aceitabilidade, a intensão de compra e as emoções evocadas durante o consumo de peitos de frango estriados.

Palavras-chave: Wooden Breast. White Striping. NIRS. Emoções. Estresse oxidativo.

ABSTRACT

The worldwide increase in the demand and consumption of chicken meat over the last decades has caused a pressure in the production systems which have tried to optimize chicken meat production. To fulfil the objective, an intense genetic selection and the improvement of animal feeding programs has led to animals with greater weight gain in a reduced period, associated with a lower cost. As a consequence of this biological pressure to grow fast, new myopathies have appeared, among them, White striping (WS) and Wooden breast (WB). The aim of this study was to analyze the occurrence of WS and WB myopathies in Northeastern Brazil, to evaluate the use of near infrared spectroscopy (NIRS) to classify breasts affected by these myopathies in an industrial slaughter plant, to investigate the effect of WS myopathy on meat quality, focusing on understanding the molecular mechanisms behind oxidative stress, and to assess how acceptability, purchase intent and emotions evoked in consumers has been affected by awareness of the causes of WS myopathy. In a first study, the occurrence of WS and WB breasts (isolated and/or combined) in birds of different age ranges (4-5, 6-7, 8-9 and 65 weeks) was verified in a commercial plant using traditional method of detection (palpation and visual aspect of the muscle). The use of near infrared spectroscopy (NIRS) associated with the SPA-LDA and SIMCA classification models was also tested as a tool to distinguish Normal (N), WS, WB and WS/WB breasts. In a second study, WS chicken breasts, with moderate and severe degrees of myopathy, were analyzed for physical-chemical properties, quality characteristics, endogenous antioxidant defenses, oxidative damage to the lipid and protein fraction, and discrimination of sarcoplasmic proteins using a Q Exactive Orbitrap mass spectrometer. In a third study, the attitude of Spanish consumers was evaluated regarding the awareness of the WS myopathy causes and consequences, and how the recognition of these breasts influences the acceptability, purchase intention and emotions evoked by consumers. The results demonstrated a high occurrence of myopathies WS, WB and WS WB in Brazil, and seem more frequent and present themselves in a more aggravated form in birds of older slaughter age. In addition, meats with myopathies presented a compromised nutritional value. The NIRS technique associated with the SPA-LDA classification model presented high accuracy in the identification of Normal (without myopathies), WS and WB (isolated and combined with WS) meats, regardless of the birds' slaughter age. Meats affected by WS showed greater depletion of free thiols, and more intense formation of malonaldehyde, allysine and Schiff base; in addition to impaired activity of endogenous antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase. The proteomic study revealed a failed attempt to maintain the biological function of muscle fiber and redox homeostasis. Striated chicken breasts (WS) showed low acceptability and purchase intent, especially when consumers were provided with information about the causes and consequences of this myopathy. The meat cooking process was able to mask the visualization of white streaks, leading consumers to have a preference for WS meat over Normal meat in unreported conditions of consumption; whereas under informed conditions, 20% of consumers tended to reject the consumption of WS meat. Furthermore, the emotional profile of consumers was significantly affected by the awareness of WS myopathies, as consumers felt more "interested" and "enthusiastic" and less "good-natured", "loving" and "warm" when consuming these meats. Based on the results generated in this research it was possible to conclude that the level of occurrence and the degree of myopathies in the breast muscle is directly related to the age of slaughter of the birds. It was also possible to confirm the promising use of the NIRS technique in the identification of myopathies in industrial processing lines. Furthermore, it showed that WS breasts present highest susceptibility to oxidative stress and, consequently, impairment of physiological and metabolic processes of the muscles. In addition, it showed that the benefit of information on what consumers eat compromise the acceptability, purchase intent and emotions evoked during consumption of striated chicken breasts.

Keyword: Wooden Breast. White Striping. NIRS. Emotions. Oxidative stress.

LISTA DE ILUSTRAÇÕES

ARTIGO III – CONSUMERS AWARENESS OF WHITE-STRIPING AS A CHICKEN BREAST MYOPATHY AFFECTS THEIR PURCHASING DECISION AND EMOTIONAL RESPONSES

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

ABPA	Associação Brasileira de Proteína Animal
CAT	Catalase
RATA	*Rate-all-that-apply*
CRA	Capacidade de retenção de água
DNPH	2,4 dinitrofenilhidrazina
DTT	Ditiotreitol
GSH-Px	Glutationa peroxidase
IAA	Iodoacetamida
LDA	Análise Linear Discriminante (*Linear discriminant analysis*)
MDA	Malonaldeído
NIRS	espectroscopia do infravermelho próximo (*Near infrared spectroscopy*)
PPGCTA	Programa de Pós-graduação em Ciência e Tecnologia de Alimentos
SIMCA	Modelagem Suave Independente por Analogia de Classe (*Soft independent modeling of class analogy)*
SM	Spaghetti Meat
SOD	Superóxido disminutase
SPA	Algoritmo das Projeções Sucessivas (*Successive projection algorithm*)
TBARS	Substâncias reativas ao ácido tiobarbitúrico
TCA	Ácido tricloroacético
TEP	1,1,3,3 tetraetoxipropano
TFA	Ácido trifluoracético
TPA	Perfil de Textura instrumental (*Texture profile analysis*)
UEx	Universidade de Extremadura
WB	Peito amadeirado (*Wooden Breast*)
WB-mod	Peito amadeirado grau moderado
WB-sev	Peito amadeirado grau severo
WS	Peito estriado (*White Striping*)
WS-mod	Peito estriado grau moderado
WS-sev	Peito estriado grau severo
WS/WB	Peito estriado e amadeirado, combinadas

SUMÁRIO

1 INTRODUÇÃO

No ano de 2019, segundo a Associação Brasileira de Proteína Animal (ABPA) a produção mundial de carne de frango superou a marca de 98 milhões de toneladas. Neste contexto, no cenário mundial, o Brasil destacou-se como terceiro maior produtor (13,2%), e maior exportador da carne de frango, sendo responsável por aproximadamente 34% do volume total exportado (ABPA, 2020).

Entre 1968 e 2018, houve uma intensa expansão da produção da carne de frango, aumentando de 9,5 para 68,8 bilhões de animais abatidos (FAOSTAT, 2019). Esta expansão foi consequência do aumento do consumo, pelo fato de ser uma proteína cárnea de baixo custo, com grande variabilidade na forma de preparo e propriedades nutricionais e dietéticas vantajosas em relação à carnes de outras espécies (PETRACCI et al., 2019). Para atender esta crescente demanda, a intensa seleção genética e os cuidados no manejo dos animais têm contribuído para o aumento da taxa de crescimento das aves, maior eficiência alimentar e maior rendimento do músculo do peito (TICKLE et al., 2014). De acordo com Zuidhof et al. (2014), entre 1957 e 2005, a taxa de crescimento de frangos de corte aumentou mais de 400%.

No entanto, por extrapolar as fronteiras biológicas dos animais, a intensa manipulação genética e alimentar, pode ter resultado no surgimento e/ou aumento de novas miopatias na carne de aves (PETRACCI e CAVANI, 2012; MALILA et al., 2018; PETRACCI et al. 2019). Conforme Sihvo et al. (2014), entre as miopatias emergentes em carne de frango, pode-se destacar o peito estriado (*White Striping*) e o peito amadeirado (*Wooden Breast*).

A miopatia denominada de *White Striping* (WS) é caracterizada pela presença de estrias brancas na superfície do músculo acompanhando a direção das fibras musculares (PETRACCI; CAVANI, 2012). Enquanto a miopatia *Wooden Breast* (WB) é marcada pela cor pálida e dureza substancial do músculo do peito, podendo apresentar focos de hemorragia e presença de exsudado na superfície do músculo (BAILEY et al., 2015). Também, sendo possível observar estas duas miopatias de maneira combinada (WS/WB).

Miopatias WS e WB têm sido relatadas em diferentes países, com valores de até 95% de ocorrência. A ocorrência de WS variou de 44-72% dos animais na Turquia (ADABI E SONCU, 2019), 48-63% nos EUA (KUTTAPPAN et al., 2009), 89-95% na Tailândia (MALILA et al., 2018) e 10-82% na Itália (PETRACCI et al., 2013; RUSSO et al, 2015). Enquanto a ocorrência de carne WB variou de 8 a 16% na Itália (TROCINO et al., 2015) e

WS/WB de 7-8% na Tailândia (MALILA et al., 2018). No entanto, ao contrário de outros países, há pouca informação disponível sobre a ocorrência de miopatias de WS e WB no Brasil.

Em geral, a identificação dessas miopatia é realizada *post-mortem* por meio de exame visual e/ou palpação do peito, requerendo um número considerável de avaliadores treinados, além de apresentar sensibilidade de detecção variável. Procurando contornar estas deficiências métodos instrumentais rápidos e não destrutivos estão sendo estudados como uma alternativa ao método tradicional de identificação.

Por serem facilmente identificadas na superfície do peito do frango, essas miopatias podem afetar diretamente a aceitação e intenção de compra dos consumidores. Kuttapan et al. (2012a) destacam que a aparência visual é o primeiro e mais importante atributo para o consumidor avaliar a qualidade da carne embalada.

Peitos de frango afetados pela miopatia WS apresentam características de qualidade alimentar prejudicadas (aparência, textura), propriedades tecnológicas ruins (menor retenção de água), valor nutricional alterado e também podem afetar a aceitação e a decisão de compra do consumidor (PETRACCI et al., 2019).

Como reflexo da miodegeneração, o aumento da deposição de gordura (lipidose) e tecido conjuntivo (fibrose) ocorre juntamente com inflamação intersticial, edema, infiltração de células inflamatórias e necrose, com aparecimento de estrias (KUTTAPPAN et al., 2013a). Mesmo com a ocorrência acentuada e pouco comum das estrias branca, observa-se que as propriedades sensoriais dos peitos de frango não parecem ser seriamente afetadas pela condição WS. No entanto, é amplamente ignorado até que ponto os consumidores de carne de frango estão cientes da origem dessas estrias ou se prestam atenção a esta aparência incomum.

Para obter mais informações sobre a atitude do consumidor em relação às miopatias emergentes de peito de frango emergentes, recomenda-se a aplicação de técnicas sensoriais inovadoras. O estudo das emoções desencadeadas pelos alimentos vem assim, atraindo recentemente considerável atenção na ciência sensorial e do consumidor.

Em uma pesquisa de revisão, Petracci et al. (2019) resumiram os potenciais mecanismos responsáveis pelo desencadeamento da miopatia WS, enfatizando que o estresse oxidativo parece desempenhar um papel central. No entanto, os mecanismos fundamentais de tais processos não são bem compreendidos. Uma melhor compreensão das bases moleculares dessa miopatia pode não apenas auxiliar no diagnóstico e na prevenção do distúrbio; como também pode permitir uma gestão mais eficiente da carne desses animais para aliviar os sintomas e melhorar a qualidade da carne.

O estresse oxidativo no tecido muscular envolve um desequilíbrio entre fatores pró-oxidantes, como espécies reativas de oxigênio, e as defesas antioxidantes, como glutationa (GSH), tocoferóis e enzimas antioxidantes (catalase, GSH-peroxidase, superóxido dismutase, entre outros) (ESTÉVEZ, 2015). O dano oxidativo às proteínas é uma característica típica desses ambientes pró-oxidativos, e sabe-se que a oxidação de proteínas leva a condições fisiológicas e doenças alteradas em animais e humanos (GARCIA-GARCIA, 2012).

A instauração do processo de oxidação proteica em carnes e produtos cárneos promove o comprometimento do valor nutricional (LUND et al., 2011) e da digestibilidade do alimento (ESTEVÉZ E LUNA, 2016) devido a degradação de aminoácidos, além da modificação da funcionalidade das proteínas (solubilidade, capacidade de retenção de água, de formação de géis e emulsões) devido a desnaturação das proteínas miofibrilares (POPOVA et al., 2009; OOIZUME; XIONG, 2008).

De acordo Halliwell et al. (1995) a oxidação lipídica produz espécies reativas (ROS) que são capazes de se ligar a importantes moléculas. Essa interação resulta na formação de compostos tóxicos ou que causam o aparecimento de sabores e odores indesejáveis (DOMÍNGUEZ et al., 2019), e compromete o valor nutritivo, a cor e a textura dos alimentos (XIAO et al., 2011). Viljanen, Kivikari e Heinonen (2004) destacaram que os produtos primários e secundários da oxidação lipídica são capazes de interagir com proteínas desencadeando a oxidação proteica; que por sua vez, pode causar a oxidação de lipídios na presença de metais.

Em virtude do músculo *Pectoralis major* de frangos ser o principal foco dos programas de seleção genética, pelo crescimento da avicultura no cenário nacional e internacional nessas últimas décadas, pelo surgimento de miopatias na carne de frango, bem como, pelo grande interesse do consumidor com a qualidade nutricional do alimento, e pelas modificações sensorias e tecnológica que a oxidação promove nos alimentos, objetivou-se: i) estudar a ocorrência das miopatias *White Striping* e *Wooden Breast* no Brasil, avaliando o uso da espectroscopia no infravermelho próximo associado a análise multivariada como ferramenta para distinguir músculos normais, WS e WB; ii) investigar os mecanismos moleculares envolvidos no aparecimento da miopatia da *White Striping* (WS), com especial atenção ao papel do estresse oxidativo e da oxidação de proteínas na perda da qualidade da carne; iii) avaliar o grau de conhecimento, aceitabilidade e intenção de compra do consumidor de peitos de frango crus e assados afetados por WS em comparação com N, antes e depois de serem informados da condição da WS.

2 REVISÃO DA LITERATURA

A revisão da literatura está apresentada sob a forma de artigo de revisão, em atendimento a Norma Complementar nº 03/2011 do PPGCTA. O artigo foi publicado no periódico *Comprehensive Reviews in Food Science and Food Safety* em 2019, sob o título *Wooden-Breast, White Striping, and Spaghetti Meat: Causes, consequences and consumer perception of emerging broiler meat abnormalities* (https://doi.org/10.1111/1541-4337.12431).

Comprehensive REVIEWS in Food Science and Food Safety

Wooden-Breast, White Striping, and Spaghetti Meat: Causes, Consequences and Consumer Perception of Emerging Broiler Meat Abnormalities

M. Petracci, F. Soglia, M. Madruga, L. Carvalho, Elza Ida, and M. Estévez

Abstract: Ten years ago, the occurrence of macroscopic defects in breasts muscles from fast-growing broilers challenged producers and animal scientists to label and characterize myopathies wholly unknown. The distinctive white striations in breasts affected by white striping disorder, the presence of out-bulging and pale areas of hardened consistency in the so-called wooden breast, and the separation of the fiber bundles in breasts labelled as spaghetti meat, made these myopathies easily identified in chicken carcasses. Yet, the high incidence of these myopathies and the increasing concern by producers and retailers led to an unprecedented flood of questions on the causes and consequences of these abnormal chicken breasts. This review comprehensively collects the most relevant information from studies aimed to understand the pathological mechanisms of these myopathies, their physicochemical and histological characterization and their impact on meat quality and consumer's preferences. Today, it is known that the occurrence is linked to fast-growth rates of the birds and their large breast muscles. The muscle hypertrophy along with an unbalanced growth of supportive connective tissue leads to a compromised blood supply and hypoxia. The occurrence of oxidative stress and mitochondrial dysfunction leads to lipidosis, fibrosis, and overall myodegeneration. Along with the altered appearance, breast muscles affected by the myopathies display poor technological properties, impaired texture properties, and reduced nutritional value. As consumer's awareness on the occurrence of these abnormalities and the concerns on animal welfare arise, efforts are made to inhibit the onset of the myopathies or alleviate the severity of the symptoms. The lack of fully effective dietary strategies leads scientists to propose whether "slow" production systems may alternatively provide with poultry meat free of these myopathies.

Keywords: animal welfare, chicken quality, oxidative stress, spaghetti meat, white striping, wooden breast

Introduction

The past two decades have witnessed an increase of consumer preference for chicken meat over other types of muscle foods. The worldwide increased consumption of chicken can be attributed to its relative low-cost, the diversity and ease of meat preparation (Wideman, O'Bryan, & Crandall, 2016), appreciated sensory properties (Petracci, Mudalal, Soglia, & Cavani, 2015), reported nutritional and dietary properties (Estévez, 2015), and the recognized positive image of white meats (of being healthy) compared with the faded image of red meats, identified as "probably carcinogenic to humans" by the IARC (IARC, 2015). Last but not least, poultry production and consumption are worldwide phenomena as chicken meat complies with most cultural and religious principles. In order to meet the growing demand and, in parallel, to optimize broiler production and increase profits, chickens have been selected for fast growth and high yields (Petracci et al., 2015). The studies carried out by Havenstein, Ferket, and Qureshi (2003) and Havenstein, Ferket, Grimes, Qureshi, and Nestor (2007) have soundly illustrated to which extent manipulation of genetics and feeds, has been able to push the biological boundaries in animal production over the last decades. The modern Ross 308 broiler fed on 2001 feeds, would reach 1.8 kg of body weight (BW) at 32 days of age with a feed conversion (FC) of 1.47, whereas the Athens-Canadian Randombred Control (ACRBC) Strain fed on 1957 feeds, would not have reached that BW until 101 days of age with a FC of 4.42 (Havenstein et al., 2003). Irrespective of the feeds, a Ross 308 broiler presents an average BW of approximately 2.4 kg (42 days) while that of ACRBC would be approximately

CRF3-2018-0221 Submitted 9/24/2018, Accepted 1/14/2019. *Authors Petracci and Soglia are with Dept. of Agricultural and Food Sciences, Alma Mater Studiorum, Univ. of Bologna, Piazza Goidanich 60, 47521, Cesena, Italy. Authors Madruga and Carvalho are with Postgraduate program in Food Science and Technology. Dept. of Food Engineering, Federal Univ. of Paraiba, João Pessoa, Paraíba, Brazil. Author Ida is with Dept. of Food Technology, Londrina State Univ., Londrina, Brazil. Author Estévez is with Meat and Meat Products Research Inst., TECAL Research Group, Univ. of Extremadura, Avda. Universidad s/n, 10003, Cáceres, Spain. Direct inquiries to author Estévez (E-mail: mariovet@unex.es).*

doi: 10.1111/1541-4337.12431

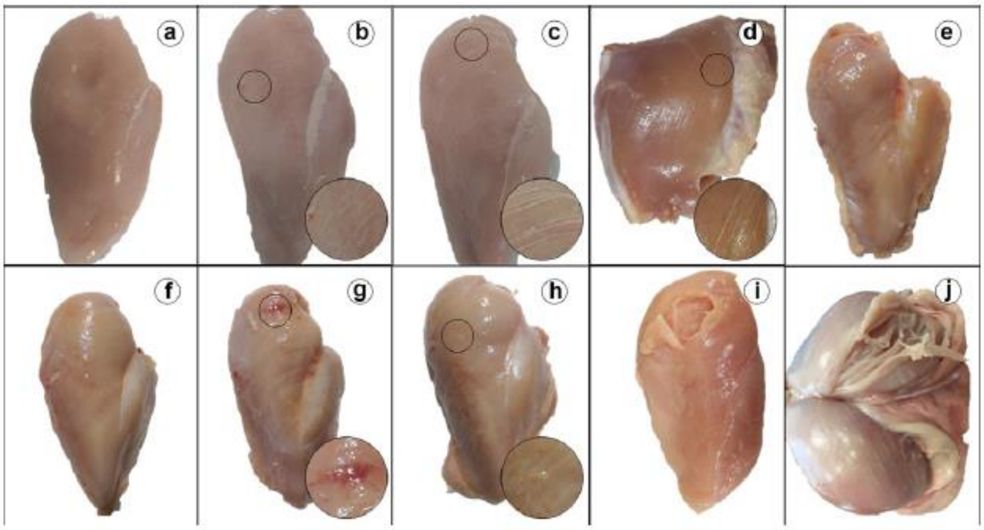


Figure 1–Classification of myopathies in chicken breast. A–Normal breast (without white striations or harden areas and hemorrhages); B–WS-moderate-2 breast (with striation < 1 mm covering the breast surface extensively); C–WS-severe-3 breast (with striation > 1 mm covering the breast surface extensively); D–WS-moderate-2 thigh (with striation <1 mm covering the thigh surface extensively); E–WB-moderate breast (with focal, hardened, and pale areas, without hemorrhages); F–WB—extremely severe breast (with diffused, hardened and pale areas, without hemorrhages); G–WB-extremely severe breast (with diffused, hardened and pale areas and hemorrhages); H–simultaneous occurrence of WS and WB (WS/WB) breast (diffused, hardened, and pale areas and superficial white striations in the cranial part); I–SM-extremely severe breast (tendency towards separation of the fiber bundles composing the muscle tissue itself).

0.55 kg at the same age (Havenstein et al., 2003). Subsequent technical guides published by Aviagen confirm the trend of increasing chicken breast yields in Ross 308 broiler (Aviagen, 2007, 2012, 2014). Comparable numbers were subsequently shown by the same authors when comparing 1966- compared with 2003-type turkeys (Havenstein et al., 2007). At the present time, those extraordinary achievements seem to have caused the arise of spontaneous myopathies in broilers and turkeys (Petracci et al., 2015) as a reflection of animal biology fighting back the human intervention. While physiological limits could have been reached already, ethical issues may have been surpassed long before as these emerging myopathies, namely white striping (WS), wooden breast (WB), and spaghetti meat (SM), are closely associated with intensive and exhausting animal production systems (Mutryn, Brannick, Fu, Lee, & Abasht, 2015; Petracci et al., 2015).

WS is easily recognized by the occurrence of white striations following the same direction of the muscle fibers in poultry breast (Figure 1). A microscopic examination of these white stripes reveals accumulation of lipids and proliferation of connective tissue (Kuttappan et al., 2013a). WB myopathy, affecting the pectoralis major and occasionally the pectoralis minor in broilers, appears as a focal lesion at approximately 2 weeks of age and subsequently develops as a widespread fibrotic injury (Papah, Brannick, Schmidt, & Abasht, 2017). The first thorough pathological description of WB was carried out by Sihvo, Immonen, and Puolanne (2014). These authors reported a hardened consistency and pale appearance in the pectoral muscles which is microscopically characterized by polyphasic myodegenerations with fibrosis in the chronic phase. Both the aforementioned abnormalities have been reported to coincide in the same muscle although the existence of concurring pathological underlying mechanisms responsible for their occurrence is a disputable topic (Cruz et al., 2016; Kuttappan, Hargis, & Owens, 2016; Livingston, Landon, Barnes, & Brake, 2018). WS, in turn, may also appear together with an additional and recently reported myopathy called SM. SM is characterized by an overall impaired integrity of the pectoralis major muscle that, exhibiting the tendency toward separation of the fiber bundles composing the muscle tissue itself, provides an image resembling the long, thin, solid, and cylindrical appearance of the popular pasta (Baldi et al., 2018). WS and WB can be classified into several grades depending on the severity of the symptoms (Figure 1).

While the information on the incidence of these myopathies is limited and, at times contradictory, it is assumed chicken breasts with abnormalities appear in all countries where fast-growing hybrids are used and that the number is higher than that the chicken industry would admit. WS, the most common of the myopathies under examination in the present review, affects to an overall level of 50% of chicken breasts in Italy, France, Spain, and Brazil (Alnahhas et al., 2016; Lorenzi, Mudalal, Cavani, & Petracci, 2014; Russo et al., 2015; Carvalho, unpublished data) with those displaying severe degree being around 20% to 30% of the total affected muscles. In Northeastern Brazil, the share of breasts with WB is reported to be between 10% and 20% with a high proportion of those breasts concurrently exhibiting WS (Carvalho, unpublished data). In Italy, a survey carried out between 2017 and 2018 on 16,000 breasts identified 42% of the samples being moderate WB and 18% being affected to a severe extent (Petracci, unpublished data). The same authors identified around 20% of the samples with SM defect. The assessment of breasts from birds at 9 weeks of age in the United States, revealed that more than 98% were found to display signs of WS (more than 55% classified as moderate and severe cases) and that around 85% of the samples displayed WB (more than 42% were severe or very severe) (Kuttappan, Owens, Coon, Hargis, & Vazquez-Anon, 2017). It should be emphasized that classification criteria may greatly vary among surveys, so absolute levels should be taken with care.

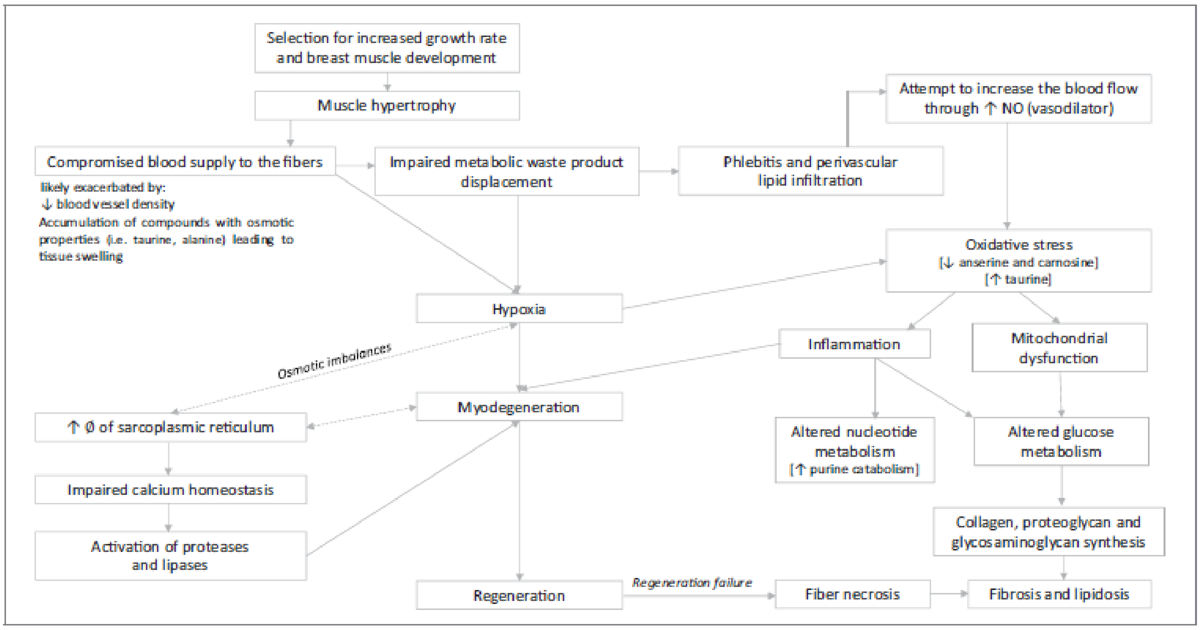


Figure 2–Schematic representation of the possible etiologies and mechanisms leading to the development of white striping (WS), wooden breast (WB), and spaghetti meat (SM) abnormalities.

These myopathies have been reported to be linked to rapid muscle growth, insufficient vascularization, and oxidative stress that may lead to tissue degeneration (Kuttappan, Brewer, Apple, Waldroup, & Owens 2012a; Soglia et al., 2016a; Papah, Brannick, Schmidt, & Abasht, 2018; Sihvo et al., 2018). Yet, and despite of the considerable efforts that have been made in the last years, the precise etiology of these abnormalities remained unclear. Regrettably, the remarkable negative impact of these muscular abnormalities on the appearance, technological and nutritional quality, and consumer acceptance of breast meat, have been clearly underlined. Hence, a profound understanding on the underlying causes and the search for solutions are needed (Baldi et al., 2018; Mutryn et al., 2015; Soglia et al., 2016a). This review compiles all relevant information in relation to the characteristics, potential etiology, quality traits, and consumer behavior toward these new myopathies and provides grounds for future challenges aimed to alleviation.

Underlying Causes of WB, WS, and SM

In the past 50 years, in order to fulfil the worldwide increasing demand for poultry meat, selection programs have been carried out to improve the production traits of broiler chickens and develop high growth-rate and breast-yield hybrids. As a consequence, outstanding results in broilers' growth performances and body composition were achieved and the number of rearing days necessary to obtain market weight birds was reduced to one-half (Petracci, Soglia, & Berri, 2017; Tallentire, Leinonen, & Kyriazakis, 2018). In spite of that upgraded production profitability, these selection practices profoundly altered muscle architecture (that is, increase muscle fiber diameter and length, increase myofiber number, reduction in capillary density, and capillary to fiber ratio) and metabolism leading to a shift toward the glycolytic pathway (Hoving-Bolin, Kranen, Klont, Gerritsen, & De Greef, 2000). Chicken breast muscles are known to be essentially glycolytic in function and nature (Papinaho, Ruusunen, Suuronen &, Fletcher, 1996). Consistently, recent studies carried out in modern broiler strains selected for rapid growth and muscle yield revealed that the pectoralis major muscles were entirely composed of type IIB fibers and the authors hypothesized whether this condition may increase their susceptibility to the development of muscular abnormalities (Petracci et al., 2017). In this context, an overall impairment in muscle regeneration in fast-growing broilers was recently demonstrated to be a consequence of a reduction in satellite cells number and in their altered abilities to proliferate and differentiate (Clark & Velleman, 2016; Daughtry et al., 2018). In addition, it was recently found a strong genetic determinism of the WS condition in fast-growing chickens ($h^2 = 0.65$; Alnahhas et al., 2016), even if Bailey, Watson, Bilgili, and Avendano (2015) previously reported lower heritability levels in two commercial pure lines of broiler chickens selected for high ($h^2 = 0.34$) or moderate ($h^2 = 0.18$) breast meat yield. Alnahhas et al. (2016) pointed also out that WS is genetically more highly related to the development of pectoralis major muscle ($r_g = +0.73$) than to the overall growth of the body ($r_g = +0.33$). Thus, being the major contributor to the changes in breast muscle development and yield, selection might be reasonably considered as the main underpinning factor responsible for the development of these muscular abnormalities. In agreement with that, a model was recently developed to determine the contribution of different growth parameters (that is, pectoralis major yield, length, depth) in predicting the severity of the myopathic lesions associated with WS and WB. The findings demonstrated that all those physical measurements inherent to genetic selection for fast-growing and high-breast yield hybrids were strictly related to the occurrence and the severity of muscular abnormalities (Griffin, Moraes, Wick, & Lilburn, 2018).

However, the studies carried out in order to quantify genes expression and identify putative causative genes leading to the development of WS (Pampouille et al., 2018) and/or WB (Abasht, Mutryn, Michalek, & Lee, 2016; Huber, Williams, & Athrey, 2018; Mutryn et al., 2015; Papah et al., 2018; Zambonelli et al., 2016) evidenced a complex etiology and a polygenic inheritance of the defect. Indeed, several genes and metabolites were differentially expressed between the pectoralis major affected by WS and/or WB and the unaffected (or only mildly affected) cases. However, since it was not possible to identify biomarkers able to discriminate WS from WB, a common etiology leading to the development of these muscular abnormalities might be reasonably hypothesized. Nevertheless, WS, WB, and SM abnormalities exhibited a distinctive phenotype that might be considered as an individual-response to the profound alteration in muscle tissue induced by genetic selection.

In this context, in the past few years, several studies were carried out in order to identify the underlying mechanisms and metabolic pathways involved in the occurrence of WS, WB, and SM muscular abnormalities (Abasht et al., 2016; Boerboom, van Kempen, Navarro-Villa, & Pérez-Bonilla, 2018; Hubert et al., 2018; Mutryn et al., 2015; Papah et al., 2017; Papah et al., 2018; Sihvo, Airas, Lindén, & Puolanne 2018; Zambonelli et al., 2016). However, the exact etiology and the chronology of events leading to the development of these defects are only partially understood and fragmentarily described. Since these muscular abnormalities exhibit similar histological features, a common underlying mechanism responsible for their occurrence might be hypothesized. A schematic representation of the underlying mechanisms leading to the progression of WS, WB, and SM abnormalities is shown in Figure 2. An early version of this illustration, reported in a previous review (Petracci et al., 2017), has been updated with findings from recent studies (Boerboom et al., 2018; Papah et al., 2017, 2018; Sihvo et al., 2018).

Selection for fast-growing and high-breast development in modern broiler hybrids, achieved through fibers' hypertrophy, likely resulted in compromised blood and oxygen supply to the muscle tissue leading to the development of hypoxia (Hoving-Bolink et al., 2000; Sihvo, Airas, Lindén, & Puolanne, 2018). This condition might even be exacerbated by the physiology of the pectoralis major muscle: its impressive development (with particular reference to thickness) might compress the pectoral artery thus further reducing oxygenation and nutrients transportation to the muscle. This hypothesis is further supported by the evidence that the severity of the histological lesions, associated with the occurrence of WS, WB, and SM, gradually decreases moving from the skin-facing surface towards the inner section of the pectoralis major (Baldi et al., 2018; Clark & Velleman, 2016; Soglia et al., 2016a). In addition, hypoxic conditions seem to be exacerbated by the reduced blood vessel density observed in WB affected muscles (Sihvo et al., 2018) as well as by tissue swelling likely due to the accumulation of compounds having osmotic properties (that is, taurine and alanine) (Boerboom et al., 2018).

Concurrently, it has been found that metabolic waste products displacement might be impaired and associated to the development of phlebitis and perivascular lipid infiltration (Papah et al., 2017; Sihvo et al., 2017). Besides the veins, evidences of vascular pathology affecting the arteries, depicted by arteriosclerosis and atherosclerosis, has been observed by analyzing the genes differentially expressed between 3-week-old unaffected birds and WB cases (Papah et al., 2018). Within this context, it has been speculated that muscle tissue tries to overcome hypoxia by increasing blood flow through the synthesis of nitric oxide that, however, might exacerbate and accelerate the development of oxidative stress. Such pro-oxidative environment would ultimately contribute to tissue inflammation and myodegeneration (Boerboom et al., 2018). Moreover, dilation of the sarcoplasmic reticulum (increased diameter), mitochondrial swelling, vacuolation, cristae loss, and mitochondrial hyperplasia (identified as early ultrastructural changes associated with the occurrence of WB) are induced by the osmotic imbalances resulting from hypoxia and myodegeneration (Sihvo et al., 2018). In addition, impaired calcium homeostasis (Boerboom et al., 2018; Mutryn et al., 2015; Zambonelli et al., 2016) may lead to the activation of proteases and lipases (Zambonelli et al., 2016) thus contributing to myodegeneration and protein degradation (Petracci et al., 2015). Then, complex biological reactions and regenerative processes, aimed at alleviating inflammation and limiting cellular apoptosis and tissue necrosis, take place (Petracci et al., 2017). When myodegeneration overtake the regenerative capacity of the muscle, altered nucleotide (as evidence by an increased purine catabolism), and carbohydrate metabolisms are observed (Abasht et al., 2016; Papah et al., 2018; Zambonelli et al., 2016) and ultimately result in fibrosis and lipidosis, distinctive microscopic traits associated with the occurrence of these growth-related abnormalities.

Physicochemical and Histological Properties of WB, WS, and SM

Classification of myopathies

The identification of myopathies in poultry breasts and establishing the degree of severity, are carried out by visual examination and/or palpation of chicken breast muscle (Figure 2) (Kuttappan et al., 2013a,b; Mutryn et al., 2015). Breasts affected by WS have been characterized by the presence of white striations parallel to muscle fibers. In the breast muscle, stripes usually appear in the cranial part of the fillet near the wing attachment and may, or may not, extend along the muscle to the caudal region (Ferreira, Casagrande, Vieira, Dreimeier, & Kindlein, 2014). Typically, breasts with WS are categorized into two grades: moderate and severe. Those with a moderate degree predominantly present striations with a thickness of less than 1 mm, while the severely affected cases, most often, have striations with a thickness greater than 1 mm; both easily visible (Kuttappan et al., 2012a,c; Kuttappan, Brewer, Apple, Waldroup, and Owens, 2012b). In addition, Bailey et al. (2015), scored WS using a three-point scale of severity: 1 (mild–focal appearance of stripes covering part of the breast), 2 (moderate—stripes extensively covering the breast surface), 3 (severe—very thick stripes with extensive coverage over the breast surface). This condition, although frequently reported in the breast meat (pectoralis major muscle), can also be seen in the thigh (iliotibialis muscle), tenders (pectoralis minor muscle), and drumsticks (gastrocnemius muscle) (Kuttappan et al., 2013c; Zimermann et al., 2012).

WB is characterized by a hardening of the breast muscle, which may have on its surface paler color, surface hemorrhaging and exudate (Kuttappan et al., 2016; Sihvo et al., 2014). This rigidity can be localized (the muscle presents focal rigid consistency, while other areas have normal consistency) or diffuse (extend throughout the breast meat) (Sihvo et al., 2018). Four categories can be attributed to WB (Papah et al., 2017; Petracci et al., 2017; Sihvo et al., 2017): mild (focally diffused and light firmness), moderate (focally diffused with extensive firmness of the breast), severe (>75% of the breast being extremely firm and with diffuse coverage) and extremely severe (firm breast). Although chicken breasts may present only symptoms from this myopathy, it is often reported to be associated with WS (Kuttappan et al., 2017; Soglia et al., 2016a). It is important to highlight that, although WB is commonly detected by manual palpation of the breast, this method may not be fully effective. Velleman, Clark, and Tonniges (2017) hypothesized that palpable hardness of the WB breast may be a result of the fibrosis resulting from the accumulation of cross-linked collagen fibrils. Other authors reported consistent results and indicated that collagen may play a major role in defining the increased firmness associated to the development of this condition (Soglia et al., 2017). It should be emphasized that the occurrence of fibrosis in WB may not necessarily affect the texture properties of the breast (Sihvo et al., 2017), and therefore, the detection by palpation may be an intricate task. WB myopathy is generally detected easier when the birds reach the age of slaughter (around 40 days) in the producing farms, or after slaughter in the slaughterhouses (Papah et al., 2017).

The condition SM is characterized by a separation of the bundles of muscle fibers mainly in the cranial region of the breast muscle and this may, or may not, be associated with WS (Baldi et al., 2018). It is still to be determined whether this histological condition occurs in the living animal.

The weakness in the technique for detecting these myopathies is a key point and fast and nondestructive methods need to be developed and applied. On this regard, some successful attempts have been made, such as the application of radiofrequency spectra in the detection of WS in skin-on chicken carcasses (Traffano-Schiffo, Castro-Giraldez, Colom, & Fito, 2017) and the usage of near-infrared spectroscopy (NIR) for the detection of breast fillets chicken with WB (Wold, Måge, Løvland, Sanden, & Ofstad, 2018; Wold, Veiseth-Kent, Høst, & Løvland, 2017). Although the use of NIR in the detection of WB was effective, the technique was developed for breast fillet, and its efficacy in carcasses was not tested. It is therefore not known to which extent skin would interfere in the detection of the myopathy, limiting the application of this technique on skin-on carcasses. Other accurate methods, such as histological or biochemical tests may require removing the animals from the slaughter line and sampling the muscles which means the destruction of the carcass. The use of biomarkers to identify myopathies in live birds by "omics" platforms using biological samples (that is, plasma) may be a highly remarkable option (further information in Section 7; Final Remarks). In addition, there is also a need to create a standard classification scale to identify progress in the degree of severity of WS, WB, and SM myopathies, which may improve current classification methods. Some recent attempts have been made on this regard (Daalgard et al., 2018)

Morphometric and histological changes in abnormal chicken breasts

Chickens with WS, WB, and SM usually present changes in morphometric measurements of the pectoralis major muscle (as briefly shown in Table 1), especially in breast weight, yield, and height. Morphometric analyses have shown that all data concerning breast dimensions (Alnahhas et al., 2016; Baldi et al., 2018; Bowker & Zhuang, 2016; Brambila, Bowker, & Zhuang, 2016; Mudalal, Babini, Cavani, & Petracci, 2014; Mudalal, Lorenzi, Soglia, Cavani, & Petracci, 2015), with the only exception of breast width (Baldi et al., 2018; Mudalal et al., 2014, 2015), were affected by WS. Data indicate that these parameters increase with the severity of striping thus confirming that the occurrence of WS is linked to thicker or heavier fillets. Similar results were reported by Dalle Zotte et al. (2017), Mudalal et al. (2015), Xing et al. (2017a), Xing, Zhao, Cai, Zhou, and Xu (2017b), and Dalgaard et al. (2018) in chicken meat affected by WB. Chatterjee, Zhuang, Bowker, Rincon, and Sanchez-Brambila (2016), Zambonelli et al. (2016), and Mudalal et al. (2015) found similar results for breasts with combined WS and WB myopathies; while Baldi et al. (2018) reported a higher average weight of breast fillet with SM and simultaneous occurrence of WS/WB and WS/SM when compared to normal one. In relation to the height of the pectoralis major muscle, chicken breasts affected by WS, WB, SM, WS/WB, and WS/SM had higher height in both the cranial and medial regions of the breast compared to those in normal chicken breasts (Baldi et al., 2018; Kuttappan et al., 2017; Mudalal et al., 2014, 2015; Zambonelli et al., 2016). However, in relation to the height in the caudal region, the WS and WS/SM presented a similar dimension to the normal group, while the SM, WB, and WS/WB breasts had a larger dimension (Baldi et al., 2018; Kuttappan et al., 2017; Mudalal et al., 2014; Mudalal et al., 2015; Zambonelli et al., 2016). No relationship was observed between breast width and myopathies (Baldi et al., 2018; Mudalal et al., 2014, 2015; Zambonelli et al., 2016), yet, a largest length in breasts was observed in WS, SM, and WS/SM affected muscles in comparison with normal breasts (Baldi et al., 2018; Mudalal et al., 2014). In WB, an accretion of extracellular material has been reported to occur in lesion areas. According to Soglia et al. (2017) and Daalgard et al. (2018) this accumulation, which may concur with water mobility, is significantly higher in breasts affected by WB than in normal ones. Therefore, the high incidence of myopathies, and particularly WB, in large breast muscles, may be a consequence of the pathological mechanisms involving edema and inflammation.

In addition to changes in size and weight of the pectoralis major muscles, degenerative lesions are also observed in WS, WB, and SM affected cases. In detail, WS exhibits an increased deposition of fat and proliferation of connective tissue within the endo- and peri-mysial compartments, loss of cross-striation, degeneration up to necrosis and regeneration of the fibers, interstitial inflammation, edema, infiltration of inflammatory cells—macrophages and lymphocytes; with the severity of the histopathological lesions gradually increasing with the severity of the striae (Kuttappan et al., 2013a,b). Sihvo et al. (2017) pointed out that broiler chickens affected by WB myopathies begin to develop morphological changes at approximately two weeks of age and, as the age increases, the lesions tend to aggravate. The most frequent changes observed in chicken breasts with WB are multifocal degeneration and necrosis, loss of striation, infiltration of macrophages and lymphocytes in the fibers, thickening of the perimysial connective tissue, fibrous connective tissue in superficial and deep part, deposit of extracellular collagen, myodegeneration, and regeneration (Sihvo et al., 2014; 2017), nuclear internalization, hyaline and vacuolar degeneration (Soglia et al., 2017), variability in shape, size, and diameter of the muscle fibers, edema, vasculitis, and perivascular infiltrations in veins (Sihvo et al., 2014, 2017). Overall, SM affected muscles display histological features similar to those previously reported in WS and WB. However, the separation of the fiber bundles composing the muscle tissue as well as the progressive degradation of the perimysial connective tissue observed by Baldi et al. (2018) can be considered distinctive microscopic features of SM defect. At last, it is worth mentioning that WS/WB and WS/SM conditions generally displayed pathological characteristics similar to those observed in muscles affected by just one myopathy.

Physical–chemical modifications in abnormal chicken breasts

Chicken breast affected by either one or two of the myopathies (WB, WS, and SM) display differences in chemical composition

Table 1–Variation in morphometric parameters of chicken breast affected by white striping (WS), wooden breast (WB), and spaghetti meat (SM) myopathies.

Morphometric parameters	Abnormality	Occurrence	References
Live weight	WS moderate	WS>N	Alnahhas et al. (2016)
		No effect	Kuttappan et al. (2012b)
	WS severe	WS>N	Alnahhas et al. (2016); Kuttappan et al. (2013b)
		No effect	Kuttappan et al. (2012b)
Breast weight	WS[c]	WS>N	Baldi et al. (2018); Mudalal et al. (2015)
	WS moderate	WS>N	Bowker and Zhuang (2016); Brambila et al. (2016)
		No effect	Kuttappan et al. (2012b)
	WS severe	WS>N	Bowker and Zhuang (2016); Brambila et al. (2016); Kuttappan et al. (2013b); Kuttappan et al. (2013b, 2012b); Mudalal et al. (2014)
	WB[d]	WB>N	Dalle Zotte et al. (2017)
	WB moderate	WB>N	Dalgaard et al. (2018)
	WB severe	WB>N	Dalgaard et al. (2018); Xing et al. (2017b); Mudalal et al. (2015)
	SM	SM>N	Baldi et al. (2018)
	WS/WB	WS/WB>N	Chatterjee et al. (2016); Zambonelli et al. (2016); Mudalal et al. (2015)
	WS/SM	WS/SM>N	Baldi et al. (2018)
Breast yield	WS moderate	WS>N	Alnahhas et al. (2016); Kuttappan et al. (2012b)
	WS severe	WS>N	Alnahhas et al. (2016); Kuttappan et al. (2012b); Kuttappan et al. (2012b, 2013b, 2017b[a])
		No effect	Kuttappan et al. (2017)[b]
	WB severe	WB>N	Kuttappan et al. (2017)[a,b]
	WS/WB	WS/WB>N	Kuttappan et al. (2017)[a,b]
Breast Length	WS[c]	WS>N	Baldi et al. (2018)
		No effect	Mudalal et al. (2015)
	WS severe	WS>N	Mudalal et al. (2014)
	WB severe	No effect	Mudalal et al. (2015)
	SM	No effect	Baldi et al. (2018)
	WS/WB	No effect	Mudalal et al. (2015); Zambonelli et al. (2016)
	WS/SM	WS/SM>N	Baldi et al. (2018)
Breast width	WS[c]	No effect	Baldi et al. (2018); Mudalal et al. (2015)
	WS severe	No effect	Mudalal et al. (2014)
	WB severe	No effect	Mudalal et al. (2015)
	SM	No effect	Baldi et al. (2018)
	WS/WB	WS/WB>N	Zambonelli et al. (2016)
		No effect	Mudalal et al. (2015)
	WS/SM	No effect	Baldi et al. (2018)
Breast top height	WS[c]	WS>N	Baldi et al. (2018); Mudalal et al. (2015)
	WS severe	WS>N	Mudalal et al. (2014), Kuttappan et al. (2017)[a]
		No effect	Kuttappan et al. (2017)[b]
	WB severe	WB>N	Kuttappan et al. (2017)[a]; Mudalal et al. (2015)
		No effect	Kuttappan et al. (2017)[b]
	SM	SM>N	Baldi et al. (2018)
	WS/WB	WS/WB>N	Kuttappan et al. (2017)[a,b]; Mudalal et al. (2015); Zambonelli et al. (2016)
	WS/SM	WS/SM>N	Baldi et al. (2018)
Breast middle height	WS[c]	WS>N	Baldi et al. (2018); Mudalal et al. (2015)
	WS severe	WS>N	Mudalal et al. (2014)
	WB severe	WB>N	Mudalal et al. (2015)
	SM	SM>N	Baldi et al. (2018)
	WS/WB	WS/WB>N	Mudalal et al. (2015); Zambonelli et al. (2016)
	WS/SM	WS/SM>N	Baldi et al. (2018)
Breast bottom height	WS[c]	No effect	Baldi et al. (2018); Mudalal et al. (2015)
	WS severe	No effect	Mudalal et al. (2014); Kuttappan et al. (2017)[b]
		WS>N	Kuttappan et al. (2017)[a]
	WB severe	WB>N	Kuttappan et al. (2017)[a,b]; Mudalal et al. (2015)
	SM	SM>N	Baldi et al. (2018)
	WS/WB	WS/WB>N	Kuttappan et al. (2017)[a,b]; Mudalal et al. (2015); Zambonelli et al. (2016)
	WS/SM	No effect	Baldi et al. (2018)

[a]Male Broiler 6 weeks-day.
[b]Male Broiler 9 weeks-day.
[c]Medium-to-thick white striations.
[d]Myopathy degree nonspecified.

when compared with normal chicken breasts (Table 2). WS exhibits higher fat and collagen contents and lower protein level compared to normal meat (Baldi et al., 2018; Kuttappan, Brewer, Apple, Waldroup, & Owens, 2012b; Malila et al., 2018; Mudalal et al., 2014; Petracci, Mudalal, Babini, & Cavani, 2014; Soglia et al., 2016a; Soglia, Laghi, Canonico, Cavani, & Petracci 2016b). The content of moisture and ashes in affected breasts vary between authors and studies (Baldi et al., 2018; Kuttappan et al., 2012b; Petracci et al., 2014; Soglia et al., 2016a,b; Soglia et al., 2018b). It is important to emphasize that although some studies show an increase in collagen content in breasts with WS, this increase is not observed in all reports. According to the literature, in

Table 2–Variation in chemical parameters of chicken breast affected by white striping (WS), wooden breast (WB), and spaghetti meat (SM) myopathies.

Chemical parameters	Abnormality	Occurrence	References
Proximate composition			
Moisture	WS[d]	No effect	Baldi et al. (2018)[a]; Soglia et al. (2016a,b)
	WS moderate	No effect	Soglia et al. (2018b)[c]; Kuttappan et al. (2012b); Petracci et al. (2014)
	WS severe	No effect	Soglia et al. (2018b)[c]; Kuttappan et al.(2012b); Petracci et al. (2014)
		WS>N	Mudalal et al. (2014)
	WB moderate	WB>N	Wold et al. (2017)
	WB severe	WB>N	Wold et al. (2017); Cai et al. (2018); Soglia et al. (2016a,b)
	SM	SM>N	Baldi et al. (2018)[a]
	WS/WB	WB>N	Soglia et al. (2016a,b)
	WS/SM	WS/SM>N	Baldi et al. (2018)[b]
Protein	WS[d]	WS<N	Baldi et al. (2018)[a]; Soglia et al. (2016b)
	WS moderate	No effect	Soglia et al. (2018b)[c] Kuttappan et al. (2012b)
		WS<N	Petracci et al. (2014)
	WS severe	No effect	Soglia et al. (2018b)[c]
		WS<N	Petracci et al. (2014); Mudalal et al. (2014); Kuttappan et al. (2012b)
	WB moderate	WB<N	Wold et al. (2017)
	WB severe	WB<N	Wold et al. (2017); Cai et al. (2018); Soglia et al. (2016a,b)
	SM	SM<N	Baldi et al. (2018)[a]
	WS/WB	WS/WB<N	Soglia et al. (2016a,b)
	WS/SM	WS/SM<N	Baldi et al. (2018)[a]
Lipid	WS[d]	WS>N	Baldi et al. (2018)[a]; Soglia et al. (2016b)
	WS moderate	No effect	Soglia et al. (2018b)[c]; Kuttappan et al. (2012b)
	WS severe	WS>N	Soglia et al. (2018b)[c]; Kuttappan et al. (2012b)
	WB moderate	No effect	Wold et al. (2017)
	WB severe	WB>N	Wold et al. (2017); Cai et al. (2018); Soglia et al. (2016a,b)
	SM	No effect	Baldi et al. (2018)[a]
	WS/WB	WS/WB>N	Soglia et al. (2016a,b)
	WS/SM	WS/SM>N	Baldi et al. (2018)[b]
Ash	WS[d]	No effect	Baldi et al. (2018)[a]
	WS moderate	No effect	Kuttappan et al. (2012b)
		WS<N	Soglia et al. (2018b)[c]
	WS severe	No effect	Kuttappan et al. (2012b)
		WS<N	Soglia et al. (2018b)[c]; Mudalal et al. (2014)
	WB severe	WS<N	Soglia et al. (2016a)
	SM	SM<N	Baldi et al. (2018)[b]
	WS/WB	WS<N	Soglia et al. (2016a)
	WS/SM	WS/SM<N	Baldi et al. (2018)[b]
Collagen	WS[d]	No effect	Baldi et al. (2018)[a]; Soglia et al. (2016b)
	WS moderate	WS>N	Petracci et al. (2014)
		No effect	Soglia et al. (2018b)[c]
	WS severe	No effect	Soglia et al. (2018b)[c]
		WS>N	Petracci et al. (2014); Mudalal et al. (2014)
	WB severe	No effect	Cai et al. (2018)
		WB>N	Soglia et al. (2016a,b)
	SM	No effect	Baldi et al. (2018)[a]
	WS/WB	No effect	Soglia et al. (2016b)
		WB>N	Soglia et al. (2016a)
	WS/SM	No effect	Baldi et al. (2018)[a]
Intramuscular fat	WS moderate	WS>N	Petracci et al. (2014)
	WS severe	WS>N	Petracci et al. (2014); Mudalal et al. (2014)
TBARS	WS[d]	No effect	Soglia et al. (2016b)
	WS moderate	No effect	Alnahhas et al. (2016)
	WS severe	No effect	Alnahhas et al. (2016)
	WB severe	WB>N	Soglia et al. (2016b)
	WS/WB	No effect	Soglia et al. (2016b)
SFA	WS[d]	WS<N	Soglia et al. (2016b)
	WS severe	WS<N	Kuttappan et al. (2012b, 2013b)
	WB severe	No effect	Soglia et al. (2016b)
	WS/WB	No effect	Soglia et al. (2016b)
MUFA	WS[d]	No effect	Soglia et al. (2016b)
	WS severe	WS>N	Kuttappan et al. (2012b, 2013b)
	WB severe	No effect	Soglia et al. (2016b)
	WS/WB	No effect	Soglia et al. (2016b)
PUFA	WS[d]	No effect	Soglia et al. (2016b)
	WS severe	No effect	Kuttappan et al. (2012b)
		WS<N	Kuttappan et al. (2013b)
	WB severe	No effect	Soglia et al. (2016b)
	WS/WB	No effect	Soglia et al. (2016b)
EPA	WS[d]	No effect	Soglia et al. (2016b)

(*Continued*)

Table 2–Continued.

Chemical parameters	Abnormality	Occurrence	References
	WS severe	No effect	Kuttappan et al. (2013b)
		WS<N	Kuttappan et al. (2012b)
	WB severe	No effect	Soglia et al. (2016b)
	WS/WB	WS/WB<N	Soglia et al. (2016b)
DPA	WS[d]	No effect	Soglia et al. (2016b)
	WS severe	WS<N	Kuttappan et al. (2012b, 2013b)
	WB severe	No effect	Soglia et al. (2016b)
	WS/WB	WS/WB<N	Soglia et al. (2016b)
DHA	WS[d]	No effect	Soglia et al. (2016b)
	WS severe	WS<N	Kuttappan et al. (2012b, 2013b)
	WB severe	No effect	Soglia et al. (2016b)
	WS/WB	No effect	Soglia et al. (2016b)
Carbonyls	WS[d]	No effect	Soglia et al. (2016b)
	WB severe	WB>N	Soglia et al. (2016b)
	WS/WB	WS/WB>N	Soglia et al. (2016b)

[a]Superficial and deep position of breast meat.
[b]Superficial position of breast meat.
[c]Turkey breast meat.
[d]Medium-to-thick white striations.

approximately 50% of the articles published so far, this increase was evidenced with the appearance and intensity of WS myopathy. As examples, Baldi et al. (2018, 2019) and Soglia et al. (2016b) reported no effect of the WS condition on collagen content. Differences were neither detected for turkeys with different degree of WS in regard to protein content and collagen (Soglia et al., 2018b). Kuttappan et al. (2012b) and Petracci et al. (2014) pointed out that the protein content decreases as the degree of WS increases from normal to severe and an opposite trend is observed for lipid content. Ash content was found to be lower in breast muscles with moderate (Soglia et al., 2018b) and severe WS (Mudalal et al., 2014; Soglia et al., 2018b). Alnahhas et al. (2016) and Soglia et al. (2016b) detected the absence of the effect of moderate and severe WS meat on the TBARS content. Although lipid content is generally higher in WS, these breasts do not seem to be more susceptible to lipid oxidation than normal ones. Moreover, higher levels of monounsaturated fatty acids and lower levels of saturated, EPA, DPA, and DHA fatty acids were observed in chicken breasts with severe WS compared to normal breasts (Kuttappan et al., 2012b; Kuttappan et al., 2013a,b).

Similar findings have been observed in chicken breast with WB myopathy (with or without WS). Several authors have reported consistent results including significant reduction in protein and ash contents and increase in moisture and lipid levels in WB muscles compared to their normal counterpart (Cai et al., 2018; Soglia et al., 2016a,b; Wold et al., 2017). The collagen content in the WB, SM, WB, WS/WB, and WS/SM presented contrasting results since some studies showed a higher content than the normal group and others did not observe a significant difference (Baldi et al., 2018; Cai et al., 2018; Soglia et al., 2016a,b). Soglia et al. (2016b) emphasized that even WB and WS/WB breasts showed no difference in saturated, monounsaturated, polyunsaturated, and DHA fatty acids if compared to normal breasts; the former were more susceptible to oxidation, with a higher level of carbonyls in WB and WS/WB breasts and a higher TBARS content in WS and WB breasts. They also reported that only breast WS/WB presented lower DPA and EPA contents. Changes in the chemical composition of WB and WS/WB meats are consistent with the replacement of fibers by adipose tissue (lipidosis) and the accretion of extracellular water as a result of edema and inflammatory processes (Clark & Velleman, 2017; Kawasaki, Iwasaki, Yamada, Yoshida, & Watanabe, 2018).

Impact of WB, WS, and SM on Meat Quality

Depending on the severity of the defects, breast affected by the aforementioned myopathies may be withdrawn from food chain. A variable share of the breasts accepted as suitable for human consumption will also account for economic losses owing to (i) condemnation/trimming (whole breast, carcass); (ii) decreased yield and value (that is, water-holding capacity [WHC], emulsifying and gelling abilities, and so on); (iii) increased need of manual sorting at deboning line (adding and training of personnel for grading/sorting); and (iv) the rejection by consumers due to undesirable sensory properties. Despite of a likely yield reduction due to increased drip and cook losses, processing altered breasts could be an alternative option to market altered breasts as fresh meat as hence, avoid a potential rejection by consumer. Kuttappan et al. (2016) roughly estimated in $200 million per year the cost of such conditions only in the United States. Given the remarkable economic loss entailed by these emerging myopathies, the characterization of their quality profile and the underlying mechanisms of such impaired traits is essential to adequately ascribe breast muscles to a suitable processing technology and minimize the negative impact of the myopathy on chicken quality and consumer acceptability. The following lines will be devoted to describing the quality of WB, WS, and SM samples in terms of functionality, sensory properties, and nutritional value. Some of these results are summarized in Table 3.

Functionality

The functional properties of meat are typically ascribed to the ability of the myofibrillar proteins to hold water, emulsify lipids and form stable gels. In turn, meat proteins functionality is dependent on their amino acid composition, their three-dimensional structure and the complex fibrillar architecture in intact muscle (Pearce, Rosenvold, Andersen, & Hopkins, 2011). Biochemical processes affecting protein composition and integrity (proteolysis, oxidation, glycation, and so on) may impair their ability to interact with other biomolecules (lipids) and water (Estévez, 2011). The first reports on the pathological conditions of WS and WB made by Kuttappan et al. (2012b) and Sihvo et al. (2014) were readily followed by the description of their impaired quality traits and particularly, of their poor ability to hold water (Petracci, Mudalal, Bonfiglio, & Cavani, 2013; Trocino et al., 2015). There is a consensus on the impaired functionality of WB and WS and that such adverse condition is

Table 3–Technological properties of chicken breast with white striping (WS), wooden breast (WB), and spaghetti meat (SM).

Technological properties	Abnormality	Occurrence	References
L[h]	WS[h]	No effect	Baldi et al. (2018); Mudalal et al. (2015); Tasoniero et al. (2016); Trocino et al. (2015)
	WS moderate	WS>N	Alnahhas et al. (2016)[b]
		No effect	Alnahhas et al. (2016)[b]; Bower and Zhuang (2016); Brambila et al. (2016); Petracci et al. (2013); Soglia et al. (2018b)[c]; Kuttappan et al. (2013c)
	WS severe	WS>N	Alnahhas et al. (2016)[b]
		No effect	Alnahhas et al. (2016)[b]; Bower and Zhuang (2016); Brambila et al. (2016); Petracci et al. (2013); Soglia et al. (2018b)[c]; Kuttappan et al. (2013c)
	WB[i]	WB>N	Dalle Zotte et al. (2017)
		No effect	Chen et al. (2018); Trocino et al. (2015)
	WB moderate	No effect	Wold et al. (2017)
	WB severe	WB>N	Cai et al. (2018); Wold et al. (2017)
		No effect	Mudalal et al. (2015); Xing et al. (2017b)
	SM	No effect	Baldi et al. (2018)
	WS/WB	WS/WB<N	Zambonelli et al. (2016)
		No effect	Chatterjee et al. (2016); Mudalal et al. (2015); Tasoniero et al. (2016)
	WS/SM	No effect	Baldi et al. (2018)
a[h]	WS[h]	No effect	Baldi et al. (2018); Mudalal et al. (2015); Tasoniero et al. (2016)
		WS>N	Trocino et al. (2015)
	WS moderate	WS>N	Petracci et al. (2013)
		No effect	Alnahhas et al. (2016)[a,b]; Bower and Zhuang (2016); Brambila et al. (2016); Soglia et al. (2018b)[c]; Kuttappan et al. (2013c)
	WS severe	WS>N	Petracci et al. (2013)
		No effect	Alnahhas et al. (2016)[a,b]; Bower and Zhuang (2016); Brambila et al. (2016); Soglia et al. (2018b)[c]; Kuttappan et al. (2013c)
	WB[i]	WB>N	Dalle Zotte et al. (2017)
		No effect	Trocino et al. (2015)
	WB moderate	No effect	Wold et al. (2017)
	WB severe	WB>N	Cai et al. (2018); Wold et al. (2017); Xing et al. (2017b)
		No effect	Mudalal et al. (2015)
	SM	No effect	Baldi et al. (2018)
	WS/WB	WS/WB>N	Chatterjee et al. (2016); Tasoniero et al. (2016)
		No effect	Mudalal et al. (2015); Zambonelli et al. (2016)
	WS/SM	No effect	Baldi et al. (2018)
b[h]	WS[h]	No effect	Baldi et al. (2018); Mudalal et al. (2015); Tasoniero et al. (2016)
		WS<N	Trocino et al. (2015)
	WS moderate	No effect	Petracci et al. (2013); Alnahhas et al. (2016)[a,b]; Bower and Zhuang (2016); Brambila et al. (2016); Soglia et al. (2018b)[c]; Kuttappan et al. (2013c)
	WS severe	WS>N	Petracci et al. (2013); Kuttappan et al. (2017)[d,e]
		No effect	Alnahhas et al. (2016)[a,b]; Bower and Zhuang (2016); Brambila et al. (2016); Soglia et al. (2018b)[c]; Kuttappan et al. (2013c)
	WB[i]	WB>N	Dalle Zotte et al. (2017)
		No effect	Trocino et al. (2015)
	WB moderate	WB>N	Wold et al. (2017)
	WB severe	WB>N	Cai et al. (2018); Kuttappan et al. (2017)[d,e]; Mudalal et al. (2015); Wold et al. (2017)
		No effect	Xing et al. (2017b)
	SM	No effect	Baldi et al. (2018)
	WS/WB	WS/WB>N	Kuttappan et al. (2017)[d,e]; Tasoniero et al. (2016)
		No effect	Chatterjee et al. (2016); Mudalal et al. (2015); Zambonelli et al. (2016)
	WS/SM	No effect	Baldi et al. (2018)
pH	WS[h]	WS>N	Mudalal et al. (2015); Trocino et al. (2015)
		No effect	Tasoniero et al. (2016)
	WS moderate	WS>N	Alnahhas et al. (2016)[b]; Brambila et al. (2016); Bower and Zhuang (2016)
		No effect	Petracci et al. (2013); Alnahhas et al. (2016)[a]; Soglia et al. (2018b)[c]; Kuttappan et al. (2013c)
	WS severe	WS>N	Petracci et al. (2013); Bower and Zhuang (2016)
		No effect	Alnahhas et al. (2016)[a,b]; Brambila et al. (2016); Soglia et al. (2018b)[c]; Kuttappan et al. (2013c, 2017)[d,e]
	WB[i]	WB>N	Dalle Zotte et al. (2017)
		No effect	Chen et al. (2018); Trocino et al. (2015)
	WB moderate	No effect	Dalgaard et al. (2018)[f]; Wold et al. (2017)

(*Continued*)

more intense at higher severity degrees of the myopathy (Tijare et al., 2016). Among the three muscle lesion types, WB displays particularly poor functionality which is manifested in low yields, impaired marinade uptake and reduced ability to hold water under both raw (increased drip loss) and heat treatment conditions (increased cooking loss) (Dalgaard et al., 2018; Dalle-Zotte et al., 2017; Kuttappan et al., 2017, Mudalal et al., 2015; Soglia et al., 2016a). Bowker, Maxwell, Zhuang, and Adhikari (2018) recently evaluated the performance of severe WB fillets during marination (0.75% NaCl/0.45 sodium phosphate) and oven-cooking (78 °C) and found that the marinade uptake and subsequent retention was lower in WB samples than in the normal counterparts. On the same line, Aguirre, Owens, Miller, and Alvarado (2018) assessed the capacity of chicken muscles affected by severe WB condition to retain marination solution injected in bulk with 15% brine (0.55% NaCl in the brine, final concentration). Twenty minutes after injection, the authors observed a lower marinade retention in severe WB samples than in normal ones (59% compared with 83% of the marinade injected) and more intense loss of water (23% compared with 20%) upon a flat-top grill cooking procedure (176 °C). Similar results were reported by Brambila, Bowker, Chatterjee, and Zhuang (2018) for severe WB muscles stored at either 4 °C or 20 °C prior to cooking at 76 °C. Regardless of storage temperature, cooking losses of WB fillets were between 8% and 10% units higher than those of normal chicken breasts. Chen et al. (2018) prepared meat batters and meatballs from normal, PSE-like and wooden chicken breasts. The poorer WHC in PSE-like and WB samples occurred along with a larger proportion and mobility of free water as measured by NIR. Xing et al. (2017a) not only reported the impaired WHC of meat batters produced from WB muscles; these authors observed inferior gelation properties in these batters compared to those produced from normal breast muscle, which formed more regular and pored networks. The same authors stated that increasing NaCl contents from 0% to 2% to 3% to 4% could improve the gelling abilities of WB batter to even compare the gel properties of normal batters (Xing, Zhao, Cai, Zhou, & Xu, 2017b). NaCl increases the ionic strength of the environment and hence, facilitates the solubility and functionality of myofibrillar proteins (Lobo et al., 2016). Nevertheless, the well-established theories explaining the relationship between pH and WHC in meat systems are not applicable to chicken breast muscles affected by the myopathies under study. A low final pH (close to the isoelectric point of muscle proteins approximately 5.0) leads to muscles with poor WHC while muscles with high final pH display better abilities to hold water (Hamm, 1961). However, the ultimate pH of WB (Kuttappan et al., 2017) and breasts affected by WS (Petracci et al., 2013) and SM (Baldi et al., 2018, 2019) is typically several decimals (0.2 to 0.4) higher than normal breast muscles and yet, their WHC is lower. The severe degeneration of the muscle tissue in these myopathies would explain the poor WHC and the lack of correspondence between this parameter and pH. On this line, Dalgaard et al. (2018) attributed to a disrupted and abnormal tissue structure the divergence between pH and WHC in WB muscles. The accretion of interstitial matter in WB myopathy (water, collagen, and proteoglycans) may also contribute to explain the water losses of these abnormal muscles upon compression and heating. At a molecular level, proteins have also been found to be affected by the underlying oxidative stress occurred during the onset of WB. Soglia et al. (2016b) found a timely connection between the extent of protein oxidation (as measured by total protein carbonyls) in WB and WB/WS samples and the proportion and mobility of extra-myofibrillar water fraction. Since the oxidative damage to meat proteins has been found to impair their ability to hold water and form emulsions and gels (Estévez, 2015; Utrera & Estévez, 2012), this condition may also contribute to impair the functionality of WB muscles. The impact of WS condition alone on meat functionality is subject of debate as contradictory results are found in literature. Kuttappan et al. (2013c) found no major effects of severe WS condition on protein functionality as measured by cooking loss. To similar conclusions came Bowker and Zhuang (2016) who observed similar myofibrillar protein solubility, water uptake, and cooking loss between WS and normal chicken breasts. Conversely, Mudalal et al. (2014) and Alnahhas et al. (2016), Petracci et al. (2013) found higher cooking losses in severe WS breasts compared to normal. Interestingly, the former authors aimed to explain the impaired WHC by reporting altered quantity (reduced concentration of myofibrillar proteins) and quality (increased extent of carbonylation) of proteins from WS muscles. Recently, Soglia et al. (2018b) characterized turkey breast muscles affected by WS condition at different degrees of severity and, despite of the differences in size and chemical composition, other quality traits, including WHC, remained unaffected by the myopathy. As hypothesized by the authors, species-specific physiological responses to the genetic selection in turkeys may explain the occurrence of WS condition in these birds with limited effects on meat quality. The limited knowledge available on the quality traits of SM was recently reported by Baldi et al. (2018) who compared this myopathy with WS. A nuclear magnetic resonance relaxation analysis revealed a higher proportion of extra-myofibrillar water in the superficial section of SM samples, which was manifested in a reduced WHC. These samples also had higher concentration of oxidized proteins than normal breast muscles supporting a likely influence of the chemical state of meat proteins on their functionality.

Sensory properties

Appearance, texture and flavor are the most appreciated quality traits by consumers of chicken meat (Carvalho et al., 2017; Estévez, 2015). The muscle tissue degeneration undergone during the onset of the myopathies under study has a straightforward influence on the relevant sensory traits, namely color and texture. The overall appearance of the breasts affected by the myopathies reported in this review is clearly altered as compared to a normal breast muscle (Figure 2) and that is a direct consequence of the histological hallmarks of each myopathy: presence of out-bulging and pale areas of hardened consistency in WB, appearance of white striations of variable thickness parallel to the muscle fibers direction in WS, and the disintegration of the muscle tissue in fiber bundles in SM. Instrumental measurement of the color of WB typically results in elevated lightness (L^*) (Dalle Zotte et al., 2017) and increased yellowness (b^*) (Kuttappan et al., 2017) with this alterations being worsened at higher degrees of severity (Tasoniero, Cullere, Cecchinato, Puolanne, & Dalle Zotte, 2016). Discoloration may also be noticeable upon cooking as recently reported by Zhuang and Bowker (2018) who found that the surface of cooked WB fillets was darker, redder, and more yellow than that of fillets without the WB condition. Also, recently, Baldi et al. (2018) observed that color modifications in WB (increased L^* and b^* values) and WS (increased b^* and reduced redness, a^*) only occurred in the superficial layer of the muscle (0.3 cm) while appearance of the muscles was normal at deeper layers.

The impact of the myopathies on the textural properties of chicken breast muscles has been profusely documented by studies applying both instrumental and sensory assessments. Sun, Koltes, Coon, Chen, and Owens (2018) found positive and significant correlations between compression force and the severity of WB condition ($r = 0.79$) in raw breast muscles, clearly incriminating the histological degeneration of muscle in its altered texture properties. Soglia et al. (2017) reported that the enlargement of extracellular matrix and fibrosis might contribute in explaining the different texture properties between superficial and deep layers in WB samples, with the superficial part exhibiting a higher amount of larger particles and a higher compression force compared to deeper layers. Aguirre et al. (2018) found correspondence between texture profile analysis (TPA) and sensory evaluation of severe WB muscles irrespective of the cooking methods applied (flat-top grill or oven, both at 176 °C). The altered muscles displayed higher scores for hardness, cohesiveness, denseness, chewiness, springiness, crunchiness and fibrousness than normal chicken breasts. Consistently, Brambila et al. (2018) found higher scores of cohesiveness and springiness in WB fillets cooked at 76 °C than normal counterparts. These authors also reported that springiness, hardness, and fibrousness were perceived differently between ventral and dorsal sections of cooked WB fillets. Using two instrumental texture techniques, namely, Meullenet-Owens Razor Shear (MORS) and TPA, Chatterjee et al. (2016) stated that fillets affected by WB condition required more force to cut through and were harder and chewier than normal chicken breast. These differences between normal and WB breast muscles were consistent regardless of the freshness (fresh compared with frozen-thawed) and the cooking (raw compared with cooked at 78 °C). Similar results were reported by Tijare et al. (2016) using the MORS technique. Conversely, Soglia et al. (2017) stated that texture differences between normal breasts and those affected by WB were mainly detected in raw meat, with the WB samples showing the highest compression values. According to the authors, the thermally labile nature of the cross-links will account for the comparable shear-force values with normal breast when measured on cooked samples, despite of the increased amount of connective tissue in the WB. When differences were detected in cooked samples, strategies have been proposed to alleviate the texture problems. Vacuum-tumbling marination lessened but not eliminated the texture differences between normal and severe WB cooked chicken, since the latter were harder, chewier and more fibrous than the former (Maxwell, Bowker, Zhuang, Chatterjee, & Adhikari, 2018). Furthermore, the impaired texture of WB muscles may also be noticeable after mincing and processing as exposed by Chen et al. (2018), who found lower instrumental hardness, cohesiveness and chewiness in cooked meatballs produced from WB as compared to those produced from normal chicken meat. A scanning electronic microscopy analysis of samples revealed that WB meatballs had networks with large aggregates and big cavities. While Chen et al. (2018) attributed these results to protein denaturation processes and lack of functional myofibrillar proteins, the oxidation state of such proteins (not measured in that study) may not be ruled out given the close relationship between the oxidative damage to proteins and meat protein aggregation and impaired functionality (Estévez, 2011; Utrera & Estévez, 2012).

The texture properties of WS breasts are not so affected as those with the WB condition (Baldi et al., 2018; Tasoniero et al., 2016). In fact, several authors found negligible effects of the WS condition on the texture of chicken (Kuttappan et al., 2013c) and turkey breasts (Soglia et al., 2018b). Other studies only reported significant differences when the most severe degrees of WS were assessed. Using the Allo-Kramer technique, Petracci et al. (2013) found lower shear-force values in severe WS samples than in normal and moderate WS breasts. In agreement, trained panelists were able to find differences only between severe WS samples and normal ones in the study carried out by Brambila et al. (2016), while moderate WS samples were identified as normal is terms of textural properties. In particular, cooked (78 °C) breast fillets affected by severe WS received higher scores for cohesiveness, hardness, and chewiness than normal and moderate WS samples. In the study of Baldi et al. (2019), SM seemed to display a softer texture after cooking which the authors attributed to a small content of collagen in these samples compared to normal muscles and breast muscles affected by WS and WB. Beyond the collagen content, several attempts to understand the underlying causes for these texture alterations have led scientists to establish connections between texture measurements and other chemical components (that is, water content and mobility) and assorted biochemical processes (that is, proteolysis and oxidation). Soglia et al. (2018a) studied proteolysis and calpain activity in WB muscles and concluded that the increased hardness in such myopathy seemed not to be exclusively attributed to differences in the proteolytic processes taking place within the *postmortem* period. Tasoniero, Bertram, Young, Dalle Zotte, and Puolanne (2017) investigated the relationship between hardness in WB and the myowater properties in these altered breast muscles using nuclear magnetic resonance. While a connection between increased muscle hardness and longer relaxation time of water trapped into the myofibrillar matrix was found, the authors concluded that the role of myowater on muscle hardness was not fully clarified by this study. In fact, changes in water compartmentalization associated to proteolysis during tenderization process in pork and sheep meat (Straadt, Rasmussen, Andersen, & Bertram, 2007), seemed not to be applicable in chicken breast muscles. Therefore, the underlying mechanisms behind the increased hardness in WB muscles seemed to be an open topic that may be covered by looking into the quantity and chemical state of meat proteins. Given the proven oxidative stress occurred in the myopathies and the close connection between protein oxidation and meat texture (Lund, Lametsch, Hviid, Jensen, & Skibsted, 2007), this is a well-deserved aspect to be examined.

Nutritional value

The impact of WB, WS, and SM conditions on the nutritional value of the chicken meat has only been partially covered. Most studies infer the nutritional value of the abnormal breast meats by interpreting the changes in the chemical composition as compared to normal chicken breast muscle. As commented in the previous section, the three myopathies have been found to display a higher amount of fat and moisture and less protein than normal breast muscles (Baldi et al., 2019; Petracci et al., 2014). This variation in proximate composition leads to higher energy content in severe WS breast muscles than in normal ones (451 compared with 421 Kj/100 g) (Petracci et al., 2014). Slight modifications in the fatty acid compositions have been reported but no variations in major nutritional indexes suggest that lipid quality is similar between normal chicken breasts and those affected by the myopathies under examination. Conversely, the protein quality is clearly worsened in breast muscles affected by myopathies due to (i) the higher collagen content and lower of myofibrillar proteins (Petracci et al., 2014) and (ii) the increased concentration of oxidized proteins as measured by the total amount of protein carbonyls (Baldi et al., 2019; Soglia et al., 2016b). Protein carbonylation is responsible for the subsequent formation of insoluble aggregates and has also been linked to an impaired *in vitro* digestibility of oxidized proteins (Estévez, 2015; Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015).

To our knowledge, the digestibility of proteins from WB, WS or SM has never been assessed so far. Finally, no harmful chemical species have been found in breast muscles affected by myopathies, so no specific chemical hazard linked to these abnormal breast muscles can be envisaged. However, the three myopathies have been associated with increased levels of lipid and protein oxidation products with some of these species (that is, MDA or protein carbonyls) being identified as potentially noxious to humans (Esterbauer, Muskiet, & Horrobin, 1993; Estévez & Luna, 2017).

Consumer Perception

Consumers are the final recipients of chicken meat and chicken products. The ultimate purpose of any livestock and agricultural production should be to fulfil consumer's demand on poultry meat in terms of safety, sensory properties, nutritional value, and animal welfare (Carvalho et al., 2017; Lusk, 2018; Magdelaine et al., 2008). However, the selection of fast-growth hybrids, the optimization of conversion rates and high yields and subsequent occurrence of the myopathies described in the present article, demonstrate that consumer's expectation and animal welfare may have not been in the focus of producers. The appearance of "altered meat" (that is, discoloration, petechiae, abnormal marbling, disintegration of meat structure, bulges) may be enough motivation for the consumer to reject breast meat affected by a myopathy. However, the awareness of the underlying causes of that altered breast meat and the identification of that product as a "sick muscle" may consolidate the consumer rejection, even if some of them would not particularly care about animal welfare. While this reasonable hypothesis needs to be proven, it is obvious that internationally recognized media has recently been informing of the occurrence of WS, their causes and consequences (The Sun, 2017). Even if the information divulged is (at times) biased and not fully scientifically legitimate, it is a fact that consumers from developed countries are increasing concerned on how livestock are farmed and meat is produced (The Independent, 2017). Consumer's demanding attitude is reflected in that fact that chicken consumers in the United States are currently willing to pay more for "slow growth chicken" meat as long as appropriate information is provided in the label (Lusk, 2018).

The most complete study of consumer's attitude toward chicken breast affected by myopathies was carried out by Kuttappan et al. (2012c) with U.S. consumers and several degrees of WS. In a hedonic assessment of overall linking for appearance, consumers' scores significantly decreased as the severity of the myopathy increased (6.9, normal; 6.1, moderate WS; and 4.5 severe WS in a 9-point scale). Overall, almost 57% of the consumers clearly disliked fillets with severe WS. The average purchase intent also significantly decreased in breast affected by WS from 3.6 (normal) to around 2.5 (WS) in a 5-point scale. When requested to explain the reasons for their preferences, consumers highlighted the higher marbling appearance in WS fillets, which may have been perceived as abnormal in chicken breast. Dalle Zotte et al. (2017) also made a worthy attempt to evaluate the impact of WB appearance on the quality that consumers may perceive. According to this study, WB myodegeneration worsens meat quality traits and the visual appearance of the affected breasts through the occurrence of bulges, petechiae, and exudate. Given that the visual aspect of WS and WB would affect product rejection at purchase (Kuttappan et al., 2012c), Tasoniero et al. (2016) suggested whether a previous cooking treatment would avoid the negative influence of macroscopic lesions on consumer's attitude. While this was efficiently accomplished, a subsequent hedonic assessment revealed that WS/WB meat received lower scores than normal chicken breasts due the toughness feeling in the mouth.

Means of Alleviation: Facts and Challenges

Given the notable incidence of WB, WS, and SM and assorted negative consequences of such myopathies on chicken quality and consumers' preferences, the search for solutions to avoid the occurrence or alleviate the symptoms has become a primary objective for animal and food scientists. To succeed in this venture, a previous identification of the pathological pathways of these myopathies is obviously required. Several studies have been conducted to ascertain the possible factors involved in the occurrence of WS, WB, and SM in fast-growing broilers (Table 4). It is widely accepted that the incidence of breast abnormalities rises with increasing slaughter weight (Cruz et al., 2016; Lorenzi et al., 2014; Papah et al., 2017), growth rate (Kuttappan et al., 2012a, 2013a, 2017; Lorenzi et al., 2014), and genetic potential for breast meat yield (Alnahhas et al., 2016; Bailey et al., 2015; Livingston et al., 2018; Lorenzi et al., 2014; Petracci et al., 2013; Trocino et al., 2015) in agreement with strong genetic correlations found by Alnahhas et al. (2016). This has been also confirmed by histological observations which evidenced that myodegeneration progress associated with the development of breast abnormalities is strictly related to age at slaughter (Griffin et al., 2018; Kawasaki et al., 2018; Papah et al., 2017; Radaelli et al., 2017; Sihvo et al., 2017) and breast growth pattern (Papah et al., 2017) (Table 5; Radaelli et al., 2017). Recently, it was also demonstrated that egg storage duration before hatching as well as manipulation of embryonic development by egg incubation temperatures and chick weight at hatching can affect muscle morphology traits and related to occurrence of breast abnormalities (Clark, Walter, & Velleman, 2017; Livingston et al., 2018). Lately, it was also hypothesized that there is progression from WS to WB with increasing age at slaughter (Griffin et al., 2018). Moreover, there are significant gender differences especially in WS and WB occurrence which appears to be higher in males (Kuttappan et al., 2013a; Lorenzi et al., 2014; Trocino et al., 2015). Otherwise, it was recently reported that SM condition is more prevalent in female birds (Soglia, Mazzoni, & Petracci, 2019).

While genetic selection and fast growth seem to be major causes for the occurrence of these myopathies, most of the studies aimed to mitigate occurrence and severity of breast abnormalities have been conducted in the field of animal nutrition (Table 4). The main strategies involved reduction of dietary intake of energy or amino acids by either feed restriction or changing feed formulation (Cruz et al., 2016; Kuttappan et al., 2013a; Livingston et al., 2019; Livingston, Ferket, Brake, & Livingston, 2019; Meloche, Fancher, Emmerson, Bilgili, & Dozier, 2018a; Sachs et al., 2018; Trocino et al., 2015,b,c,d; Zampiga et al., 2018). However, overall a reduction in the occurrence of breast muscle abnormalities was observed almost exclusively as an indirect result of decreased growth rate, slaughter weight, and/or breast yield (Cemin et al., 2018; Cruz et al., 2016; Livingston et al., 2018; Meloche, Dozier, Brandebourg, & Starkey, 2018d; Sachs et al., 2018). Only a minimal dietary feed restriction (95%) and short-term reduction of lysine have allowed to slightly reduce severity of WS and WB, but adoption of these strategies under commercial condition are challenging (Meloche et al., 2018d). Indeed, compensatory growth following early feed restriction may even increase incidence of breast abnormalities (Trocino et al., 2015). Analogously, different coccidiosis control approaches (Dalle Zotte, Tasoniero, Russo, Longoni, & Cecchinato, 2015) and dietary supplementation with

Table 4–Factors affecting occurrence of white striping (WS), wooden breast (WB), and spaghetti meat (SM) in fast-growing broilers.

Factor	Abnormality	Occurrence	References
Bird and live growth factors			
Strain	WS	High > Standard breast-yield	Petracci et al. (2013)
	WS		Lorenzi et al. (2014)
	WS/WB		Bailey et al. (2015)
	WS/WB		Trocino et al. (2015)
	WS/WS		Alnahhas et al. (2016)
			Livingston et al. (2018)
Egg storage	WS/WB	Long > Short (only on WS in feed restricted birds)	Livingston et al. (2018)
Gender	WS	Males > Females	Kuttappan et al. (2013a)
	WS		Lorenzi et al. (2014)
	WS/WB		Trocino et al. (2015)
Body weight/age at slaughter	WS	High > Low	Lorenzi et al. (2014)
	WS/WB		Cruz et al. (2016)
	WB		Papah et al. (2018)
Growth rate	WS	Fast > Slow	Lorenzi et al. (2014)
	WS/WB		Kuttappan et al. (2012a, 2013a, 2017)
Dietary means			
Phase-feeding	WS	No effect	Kuttappan et al. (2013a)
Early dietary restriction	WS/WB	No effect	Trocino et al. (2015)
Dietary restriction (95% freely)	WS/WB	Freely > Feed restricted	Meloche et al. (2018a)
Dietary restriction (90% freely)	WS/WB	Freely > Feed restricted[a]	Livingston et al. (2018)
Dietary energy and/or amino acid density	WS/WB	No effect	Meloche et al. (2018c)
			Bodle et al. (2018)
Lysine	WS/WB	High > Low[a]	Cruz et al. (2016)
			Meloche et al. (2018d)
Lysine (short-term reduction)	WS/WB	Moderate effect on WS/WB severity[a]	Meloche et al. (2018b)
Arginine:lysine	WS/WB	No effect	Bodle et al. (2018)
	WS/WB/SM	Low > High	Zampiga et al. (2018)
Arginine	WS/WB	High > Low[a]	Livingston et al. (2018)
Glutamine	WS/WB	High > Low[a]	Livingston et al. (2019)
Methionine	WS	Synthetic > Natural source[a]	Sachs et al. (2018)
Guanidinoacetic acid	WS/WB/SM	Slight effect on WB severity	Cordoba-Noboa et al. (2018a,b)
Vitamin E	WS	No effect	Kuttappan et al. (2012b)
Vitamin C	WS/WB	High > low[a]	Bodle et al. (2018)
Vitamin premix	WS/WB	No effect	Bodle et al. (2018)
Selenium	WB	No effect	Sihvo et al. (2017)
	WS/WB	Organic > Inorganic[a]	Cemin et al. (2018)
Organic trace minerals	WS/WB/SM	No effect	Sirri et al. (2016)
Coccidiosis control	WS	No effect	Dalle Zotte et al. (2015)
Postmortem factors			
Chilling time	WS	No effect	Kuttappan et al. (2013a)

[a]Changes result as indirect result of reduced growth rate, slaughter weight and/or breast yield.

Table 5–Factors affecting myodegeneration associated with occurrence of emerging abnormalities in fast-growing broilers.

Factor	Degree of myodegeneration	References
Egg incubation temperature	High > Standard[a]	Clark et al. (2017)
Hatching time	Early > Late[a]	Clark et al. (2017)
Gender	No effect	Radaelli et al. (2017)
Early dietary restriction	Freely > Feed restricted	Radaelli et al. (2017)
Body weight/age at slaughter	Increased with age/body weight	Radaelli et al. (2017), Sihvo et al. (2017), Kawasaki et al. (2018), Griffin et al. (2018), Papah et al. (2018)
Breast yield/size	Increased with pectoralis major yield/size	Radaelli et al. (2017) Papah et al. (2018)

[a]Changes result as indirect result of reduced growth rate, slaughter weight and/or breast yield.

antioxidants (vitamin E, C, and selenium) and organic trace minerals (Kuttappan et al., 2012b; Sihvo et al., 2017; Sirri et al., 2016), which overall have been supposed to reduce muscle fiber oxidative stress associated with breast abnormality progress, did not result in any true mitigation effects. On the other hand, a slight reduction of moderate cases of WB has been obtained by dietary supplementation of guanidinoacetic acid used a precursor of creatine, but strong interaction with diet composition was found (Cordoba-Noboa et al., 2018a,b). Recently, it was demonstrated that an increase of arginine:lysine ratio can have a mitigation effect on breast meat abnormalities (Zampiga, Soglia, Petracci, Meluzzi, & Sirri, 2018) though a similar study led to a divergent outcome (Bodle et al., 2018). Therefore, until now it seems that there is lack of practical nutrition and management interventions to reduce growth-related abnormalities in the broiler industry without negatively affecting live and slaughter performances. Finally, few studies were conducted to ascertain influence of slaughter phases (Kuttappan et al., 2013a) which anyway appear to be of little

Table 6–Processing solutions for alleviating meat quality consequences of occurrence of white striping (WS), wooden breast (WB), and spaghetti meat (SM).

Abnormality	Meat processing solutions	Effectiveness	References
WB WS/WB/SM WB	Breast fillet portioning (Separation of dorsal and ventral portions)	high	Bowker and Zhuang (2016) Baldi et al. (2018) Bowker et al. (2018)
WS WS/WB WS/WB WS/WB WB WB	Vacuum tumbling (Whole muscle)	poor	Petracci et al. (2013) Mudalal et al. (2015) Soglia et al. (2016a)) Tijare et al. (2016) Bowker et al. (2018) Maxwell et al. (2018)
WB	Coarsely mincing (Cooked patties)	high	Sanchez-Brambila et al. (2017, 2018)
WB	Finely mincing (Meat batters and meat balls)	High Poor	Xing et al. (2017b) Chen et al. (2018)

importance in determining breast abnormalities (Petracci et al., 2015).

Alternatively, some authors proposed meat processing solutions to minimize implications on product quality as summarized in Table 6. There are margins to mitigate undesirable effects on technological properties of processed products when abnormal meats are included in the formulation of grounded and finely comminuted meat products (Brambila et al., 2018; Brambila, Chatterjee, Bowker, & Zhuang, 2017, Chen et al., 2018; Xing et al., 2017a), however significant quality reduction can arise when high-quality processed products (that is, enhanced whole-muscle and ground products) are manufactured by using especially WB abnormal raw meat (Bowker, Maxwell, Zhuang, & Adhikari, 2018; Maxwell et al., 2018; Mudalal et al., 2015; Petracci et al., 2013; Soglia et al., 2016a; Tijare et al., 2016). In addition, latest studies agree that overall manifestation of muscle abnormalities mainly affects the superficial section of pectoralis major muscle, while the deep section was less affected (Baldi et al., 2018, 2019; Bowker & Zhuang, 2016; Bowker et al., 2018). Therefore, one possible strategy to optimize its use after downgrading could be to separately process the superficial (ventral) and deep (dorsal) layer of the abnormal pectoral muscle for exploiting their distinctive traits (Baldi et al., 2018, 2019; Bowker et al., 2018).

Final Remarks

The final words from this comprehensive review will be devoted to identifying unresolved problems and potential fields of study. The poultry sector is nowadays in continuous evolution in relation to the needs from the industry and the society. It seems that the poultry industry as a whole (not only breeding companies) can no longer postpone long-anticipated decision-makings and confront the awkward triangle formed by the selection of meat-type genotypes, fast muscle growth, and impaired quality in chicken breasts. Scientists, on the other hand, endure in their commitment in searching for means of alleviation. Apparently, no efficient solutions have been identified and hence, controlling the high incidence of these myopathies is a major commitment for animal scientists. A profound understanding of the molecular basis of the underlying pathological processes is essential to establish well-reasoned and reliable strategies. On this line, the application of advanced methodologies based on omics platforms are of great assistance. The study of the proteome in breasts affected by quality defects (Schilling et al., 2017) and the metabolomic studies carried out by Abasht et al. (2016) and Boerboom et al. (2018) in WB and WS, respectively, contributed to establish unique proteomic and metabolomic profiles that may be employed for diagnosis purposes and ultimately, for a better comprehension of the mechanistic insight into these myopathies. Likewise, the application of genetic-based analysis is of interest given the clear genetic determinism of some of these myopathies (Alnahhas et al., 2016). In this regard, Pampouille et al. (2018) recently carried out a genome-wide association study in which a quantitative trait loci (QTL) mapping in WS revealed a polygenic inheritance of the defect and identified several candidate genes implicated in the muscular dystrophies. In addition, Hubert, Williams, and Athrey (2018) by comparing birds having fast- and slow-growth genetic backgrounds stated that WB is a potentially polygenic, complex syndrome, with molecular similarities to neoplastic disorders. Vignale et al. (2017) contributed to understanding the impair protein and fat metabolism in chickens affected by WS analyzing the expression of genes such as MuRF1, atrogin-1, IGF-1, insulin receptor (IR), fatty acid synthetase, and acetyl CoA carboxylase (ACC).

A fast progress is envisaged in the upcoming years and answers to some unresolved questions may be found. Yet, some current and urgent challenges are calling for attention such as the management of the high numbers of chicken breast muscles affected by WS, WB, and SM. One major issue is the early detection and objective classification of the myopathies using nondestructive techniques. Aforementioned efforts have been made using radiofrequency spectra (Traffano-Schiffo et al., 2017) and NIR to detect and grade WB and WS myopathies. Further advanced options are available as the fusion of Optical Coherence Tomography (OCT) and hyperspectral imaging proposed by Yoon, Bowker, and Zhuang (2017) or other imaging techniques already used for an efficient non-destructive assessment of meat products such as ultrasounds (Corona, García-Pérez, Santacatalina, Ventanas, & Benedito, 2014) and Magnetic Resonance Imaging (MRI) (Caballero et al., 2018). Once the carcasses are identified as affected by the myopathies in a particular degree, decisions on the fate of these chicken meats have to be taken. As consumers' awareness on the occurrence and origin of these chicken defects is increasing, transforming abnormal breasts into processed chicken products may be a feasible option. However, means to improve their technological, sensory, and nutritional properties are also required. The increase in breast meat abnormalities is favoring the transition from standard chicken to differentiated/price-premium products (that is, animal welfare friendly, environment friendly, consumer health, buy local, and so on). In this unstable context, innovative strategies are also required to continuously adapt to this mutable scenario and for this reason, know-how concerning the whole production chain (from farm to fork) is more crucial than ever.

Acknowledgments

Marta Madruga, Leila Carvalho, and Elza Ida acknowledge the CNPq- Conselho Nacional de Desenvolvimiento Cientifico e Tecnológico (430832/2016-8). Mario Estévez acknowledge the CNPq and the Spanish Ministry of Economics and Competitiveness (SMEC) (projects 474300/2011-0 and AGL2017-84586-R, respectively).

Authors' Contributions

Petracci, Soglia, Madruga, Carvalho, Ida, and Estévez compiled information, interpreted results, and contributed to drafting the manuscript. Estévez and Petracci conceived and drafted the structure of the review and finalized the manuscript.

References

Abasht, B., Mutryn, M. F., Michalek, R. D., & Lee, W. R. (2016). Oxidative stress and metabolic perturbations in wooden breast disorder in chickens. *PLoS One*, *11*, 4, e0153750. https://doi.org/10.1371/journal.pone.0153750

Aguirre, M. E., Owens, C. M., Miller, R. K., & Alvarado, C. Z. (2018). Descriptive sensory and instrumental texture profile analysis of woody breast in marinated chicken. *Poultry Science*, *97*(4), 1456–1461. https://doi.org/10.3382/ps/pex428

Alnahhas, N., Berri, C., Chabault, M., Chartrin, P., Boulay, M., Bourin, M. C., & Le Bihan-Duval, E. (2016). 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*, *17*(1), 61. https://doi.org/10.1186/s12863-016-0369-2

Aviagen (2007). *Ross 308 broiler performance objectives. In-house publication, global.* Newbridge, UK: Aviagen Ltd.

Aviagen (2012). *Ross 308 broiler performance objectives. In-house publication, global.* Newbridge, UK: Aviagen Ltd.

Aviagen (2014). *Ross 308 broiler performance objectives. In-house publication, global.* Newbridge, UK: Aviagen Ltd.

Bailey, R. A., Watson, K. A., Bilgili, S. F., & Avendano, S. (2015). The genetic basis of pectoralis major myopathies in modern broiler chicken lines. *Poultry Science*, *94*(12), 2870–2879. https://doi.org/10.3382/ps/pev304

Baldi, G., Soglia, F., Mazzoni, M., Sirri, F., Canonico, L., Babini, E., . . . Petracci, M. (2018). Implications of White Striping and Spaghetti Meat abnormalities on meat quality and histological features in broilers. *Animal*, *12*(1), 164–173. https://doi.org/10.1017/S1751731117001069

Baldi, G., Soglia, F., Laghi, L., Tappi, S., Rocculi, P., Tavaniello, S., . . . Petracci, M. (2019). Comparison of quality traits among chicken breast meat affected by current muscle abnormalities. *Food Research International*, *115*, January, 369–376. https://doi.org/10.1016/j.foodres.2018.11.020.

Bodle, B. C., Alvarado, C., Shirley, R. B., Mercier, Y., & Lee, J. T. (2018). Evaluation of different dietary alterations in their ability to mitigate the incidence and severity of woody breast and white striping in commercial male broilers. *Poultry Science*, *97*(9), 3298–3310.

Boerboom, G., van Kempen, T., Navarro-Villa, A., & Pérez-Bonilla, A. (2018). Unraveling the cause of white striping in broilers using metabolomics. *Poultry Science*, *97*(11), 3977–3986. https://doi.org/10.3382/ps/pey266.

Bowker, B., & Zhuang, H. (2016). Impact of white striping on functionality attributes of broiler breast meat. *Poultry Science*, *95*(8), 1957–1965. https://doi.org/10.3382/ps/pew115

Bowker, B. C., Maxwell, A., Zhuang, D H., & Adhikari K. (2018). Marination and cooking performance of portioned broiler breast fillets with the wooden breast condition. *Poultry Science*, *97*, 2966–2970. https://doi.org/10.3382/ps/pey144

Brambila, G. S., Bowker, B. C., & Zhuang, H. (2016). Comparison of sensory texture attributes of broiler breast fillets with different degrees of White Striping. *Poultry Science*, *95*, 2472–2476. https://doi.org/10.3382/ps/pew165

Brambila, G. S., Chatterjee, D., Bowker, B., & Zhuang, H. (2017). Descriptive texture analyses of cooked patties made of chicken breast with the woody breast condition. *Poultry Science*, *96*(9), 3489–3494. https://doi.org/10.3382/ps/pex118

Brambila, G. S., Bowker, B. C., Chatterjee, D., & Zhuang, H. (2018). Descriptive texture analyses of broiler breast fillets with the wooden breast condition stored at 4°C and −20°C. *Poultry Science*, *97*, 1762–1767. https://doi.org/10.3382/ps/pew327

Carvalho, R. H., Ida, E. I., Madruga, M. S., Martínez, S. L., Shimokomaki, M., & Estévez, M. (2017). Underlying connections between the redox system imbalance, protein oxidation and impaired quality traits in pale, soft and exudative (PSE) poultry meat. *Food Chemistry*, *215*, 129–137. https://doi.org/10.1016/j.foodchem.2016.07.182

Caballero, D., Antequera, T., Caro, A., Amigo, J. M., ErsbØll, B.K, Dahl, A. B., & Pérez-Palacios, T. (2018). Analysis of MRI by fractals for prediction of sensory attributes: A case study in loin. *Journal of Food Engineering*, *227*, 1–10. https://doi.org/10.1016/j.jfoodeng.2018.02.005

Cai, K., Shao, W., Chen, X., Campbell, Y. L., Nair, M. N., Suman, S. P., . . . Schilling, M. W. (2018). Meat quality traits and proteome profile of woody broiler breast (*Pectoralis Major*) meat. *Poultry Science*, *97*(1), 337–346. https://doi.org/10.3382/ps/pex284

Cemin, H. S., Vieira, S. L., Stefanello, C., Kindlein, L., Ferreira, T. Z., & Fireman, A. K. (2018). Broiler responses to increasing selenium supplementation using Zn-L-selenomethionine with special attention to breast myopathies. *Poultry Science*, *97*(5), 1832–1840. https://doi.org/10.3382/ps/pey001

Chatterjee, D., Zhuang, H., Bowker, B. C., Rincon, A. M., & Sanchez-Brambila, G. (2016). Instrumental texture characteristics of broiler pectoralis major with the wooden breast condition. *Poultry Science*, *95*(10), 2449–54. https://doi.org/10.3382/ps/pew204

Chen, H., Wang, H., Qi, J., Wang, M., Xu, X., & Zhou, G. (2018). Chicken breast quality – Normal, pale, soft and exudative (PSE) and woody – Influences the functional properties of meat batters. *International Journal of Food Science and Technology*, *53*, 654–664. https://doi.org/10.1111/ijfs.13640

Clark, D. L., & Velleman, S. G. (2016). Spatial influence on breast muscle morphological structure, myofiber size, and gene expression associated with the wooden breast myopathy in broilers. *Poultry Science*, *95*(12), 2930–2945. https://doi.org/10.3382/ps/pew243

Clark, D. L., & Velleman, S. G. (2017). Physiology and reproduction: Spatial influence on breast muscle morphological structure, myofiber size, and gene expression associated with the wooden breast myopathy in broilers. *Poultry Science*, *95*(12), 2930–2945. https://doi.org/10.3382/ps/pew243

Clark, D. L., Walter, K. G., & Velleman, S. G. (2017). Incubation temperature and time of hatch impact broiler muscle growth and morphology. *Poultry Science*, *96*(11), 4085–4095. https://doi.org/10.3382/ps/pex202

Córdova-Noboa, H. A., Oviedo-Rondón, E. O., Sarsour, A. H., Barnes, J., Ferzola, P., Rademacher-Heilshorn, M., & Braun, U. (2018a). Performance, meat quality, and pectoral myopathies of broilers fed either corn or sorghum based diets supplemented with guanidinoacetic acid. *Poultry Science*, *97*(7), 2479–2493. https://doi.org/10.3382/ps/pey096

Córdova-Noboa, H. A., Oviedo-Rondón, E. O., Sarsour, A. H., Barnes, J., Sapcota, D., López, D., . . . Braun, U. (2018b). Effect of guanidinoacetic acid supplementation on live performance, meat quality, pectoral myopathies and blood parameters of male broilers fed corn-based diets with or without poultry by-products. *Poultry Science*, *97*(7), 2494–2505. https://doi.org/10.3382/ps/pey097

Corona, E., García-Pérez, J. V., Santacatalina, J. V., Ventanas, S., & Benedito, J. (2014). Ultrasonic characterization of pork fat crystallization during cold storage. *Journal of Food Science*, *79*, E828–E838. https://doi.org/10.1111/1750-3841.12410

Cruz, R. F. A., Vieira, S. L., Kindlein, L., Kipper, M., Cemin, H. S., & Rauber, S. M. (2016). Occurrence of white striping and wooden breast in broilers fed grower and finisher diets with increasing lysine levels. *Poultry Science*, *96*(2), 501–510. https://doi.org/10.3382/ps/pew310

Dalgaard, L. B., Rasmussen, M. K., Bertram, H. C., Jensen, J. A., Møller, H. S., Aaslyng, M. D., . . . Young, J. F. (2018). Classification of Wooden Breast myopathy in chicken pectoralis major by a standardised method and association with conventional quality assessments. *International Journal of Food Science & Technology*, *53*(7), 1–9. https://doi.org/10.1111/ijfs.13759

Dalle Zotte, A., Tasoniero, G., Russo, E., Longoni, C., & Cecchinato, M. (2015). Impact of coccidiosis control program and feeding plan on white striping prevalence and severity degree on broiler breast fillets evaluated at three growing ages. *Poultry Science*, *94*(9), 2114–2123. https://doi.org/10.3382/ps/pev205

Dalle Zotte, A., Tasoniero, G., Puolanne, A., Remignon, H., Cecchinato, M., Catelli, E., & Cullere, M. (2017). Effect of 'Wooden Breast' appearance on poultry meat quality, histological traits, and lesions characterization. *Czech Journal of Animal Science*, *62*(2), 51–57. https://doi.org/10.17221/54/2016-CJAS

Daughtry, M.R., Berio, E., Shen, Z., Suess, E. J. R., Shah, N., Geiger, A. E., . . . Gerrard, D. E. (2018). Satellite cell-mediated breast muscle regeneration decreases with broiler size. *Poultry Science*, *96*(9), 3457–3464. https://doi.orghttps://doi.org/10.3382/ps/pex068

Esterbauer H, Muskiet F., & Horrobin DF. (1993). Cytotoxicity and genotoxicity of lipid-oxidation products. *American Journal of Clinical Nutrition*, *57*, 779S–786S. https://doi.org/10.1093/ajcn/57.5.779S

Estévez, M. (2011). Protein carbonyls in meat systems: A review. *Meat Science*, *89*, 259–279. https://doi.org/10.1016/j.meatsci.2011.04.025

Estevez, M. (2015). Oxidative damage to poultry: From farm to fork. *Poultry Science*, *94*, 1368–1378. https://doi.org/10.3382/ps/pev094

Estévez, M., & Luna, C. (2017). Dietary protein oxidation: A silent threat to human health? *Critical Reviews in Food Science and Nutrition*, *57*(17), 3781–3793. https://doi.org/10.1080/10408398.2016.1165182

Ferreira, T. Z., Casagrande, R. A., Vieira, S. L., Dreimeier, D., & Kindlein, L. (2014). An investigation of a reported case of white striping in broilers. *Journal Applied Poultry Research*, *23*, 1–6. https://doi.org/10.3382/japr.2013-00847

Griffin, J. R., Moraes, L., Wick, M., & Lilburn, M. S. (2018). Onset of white striping and progression into wooden breast as defined by myopathic changes underlying pectoralis major growth. Estimation of growth parameters as predictors for stage of myopathy progression. *Avian Pathology*, *47*(1), 2–13. https://doi.org/10.1080/03079457.2017.1356908

Hamm, R. (1961). Biochemistry of meat hydration. *Advances in Food Research*, *10*(C), 355–463. https://doi.org/10.1016/S0065-2628(08)60141-X

Havenstein, G. B., Ferket, P. R., & Qureshi, M. A. (2003). Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. *Poultry Science*, *82*, 1500–1508. https://doi.org/10.1093/ps/82.10.1500

Havenstein, G. B., Ferket, P. R., Grimes, J. L., Qureshi, M. A., & Nestor, K. E. (2007). Comparison of the performance of 1966- versus 2003-type Turkeys when fed representative 1966 and 2003 Turkey diets: Growth rate, livability, and feed conversion. *Poultry Science*, *86*(2), 232–240. https://doi.orghttps://doi.org/10.1093/ps/86.2.232

Hoving-Bolink, A. H., Kranen, R. W., Klont, R. E., Gerritsen, C. L. M., & De Greef, K. H. (2000). Fibre area and capillary supply in broiler breast muscle in relation to productivity and ascites. *Meat Science*, *56*(4), 397–402. https://doi.org/10.1016/S0309-1740(00)00071-1

Hubert, S. M., Williams, T. J., & Athrey, G. (2018). Insights into the molecular basis of wooden breast based on comparative analysis of fast-and slow-growth broilers. *bioRxiv*, 356683. https://doi.org/10.1101/356683

IARC (2015). Carcinogenicity of consumption of red and processed meat. *The Lancet*, *16*, 1599–1600. https://doi.org/10.1016/S1470-2045(15)00444-1

Kawasaki, T., Iwasaki, T., Yamada, M., Yoshida, T., & Watanabe, T. (2018). Rapid growth rate results in remarkably hardened breast in broilers during the middle stage of rearing: A biochemical and histopathological study. *PloS One*, *13*(2), e0193307. https://doi.org/10.1371/journal.pone.0193307

Kuttappan, V. A., Goodgame, S. D., Bradley, C. D., Mauromoustakos, A., Hargis, B. M., Waldroup, P. W., & Owens, C. M. (2012a). Effect of different levels of dietary vitamin E (DL-α-tocopherol acetate) on the occurrence of various degrees of white striping on broiler breast fillets. *Poultry Science*, *91*(12), 3230–3235. https://doi.org/10.3382/ps.2012-02506

Kuttappan, V. A., Brewer, V. B., Apple, J. K., Waldroup, P. W., & Owens, C. M. (2012b). Influence of growth rate on the occurrence of white striping in broiler breast fillets. *Poultry Science*, *91*(10), 2677–2685. https://doi.org/10.3382/ps.2012-02259

Kuttappan, V. A., Lee, Y. S., Erf, G. F., Meullenet, J. F. C., Mckee, S. R., & Owens, C. M. (2012c). Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. *Poultry Science*, *91*, 1240–1247. https://doi.org/10.3382/ps.2011-01947

Kuttappan, V. A., Brewer, V. B., Mauromoustakos, A., McKee, S. R., Emmert, J. L., Meullenet, J. F., & Owens, C. M. (2013a). Estimation of factors associated with the occurrence of white striping in broiler breast fillets. *Poultry Science*, *92*(3), 811–819. https://doi.org/10.3382/ps.2012-02506

Kuttappan, V. A., Huff, G. R., Huff, W. E., Hargis, B. M., Apple, J. K., Coon, C., & Owens, C. M. (2013b). Comparison of hematologic and serologic profiles of broiler birds with normal and severe degrees of white striping in breast fillets. *Poultry Science*, *92*, 339–345. https://doi.org/10.3382/ps.2012-02647

Kuttappan, V. A., Shivaprasad, H. l., Shaw, D. P., Valentine, B. A., Hargis, B. M., Clark, F. D., . . . Owens, C. M. (2013c). Pathological changes associated with White Striping in broiler breast muscles. *Poultry Science*, *92*(2), 331–338. https://doi.org/10.3382/ps.2012-02646

Kuttappan, V. A., Hargis, B. M., & Owens, C. M. (2016). White striping and woody breast myopathies in the modern poultry industry: A review. *Poultry Science*, *95*(11), 2724–2733. https://doi.org/10.3382/ps/pew216

Kuttappan, V. A., Owens, C. M., Coon, C., Hargis, B. M., & Vazquez-Anon, M. (2017). Incidence of broiler breast myopathies at 2 different ages and its impact on selected raw meat quality parameters. *Poultry Science*, *96*(8), 3005–3009. https://doi.org/10.3382/ps/pex072

Livingston, M. L., Landon, C., Barnes, H. J., & Brake, J. (2018). White striping and wooden breast myopathies of broiler breast muscle is affected by time-limited feeding, genetic background, and egg storage. *Poultry Science*, Advance online publication. https://doi.org/10.3382/ps/pey333.

Livingston, M. L., Ferket, P. R., Brake, J., & Livingston, K. A. (2019). Dietary amino acids under hypoxic conditions exacerbates muscle myopathies including wooden breast and white stripping. *Poultry Science*, Advance online publication. https://doi.org/10.3382/ps/pey463.

Lobo, F., Ventanas, S., Morcuende, D., & Estévez, M. (2016). Underlying chemical mechanisms of the contradictory effects of NaCl reduction on the redox-state of meat proteins in fermented sausages. *LWT*, *69*, 110–116. https://doi.org/10.1016/j.lwt.2016.01.047

Lorenzi, M., Mudalal, S., Cavani, C., & Petracci, M. (2014). Incidence of white striping under commercial conditions in medium and heavy broiler chickens in Italy. *Journal of Applied Poultry Research*, *23*(4), 754–758. https://doi.org/10.3382/japr.2014-00968

Lund, M. N., Lametsch, R., Hviid, M. S., Jensen, O. N., & Skibsted, L. H. (2007). High-oxygen packaging atmosphere influences protein oxidation and tenderness of porcine longissimus dorsi during chill storage. *Meat Science*, *77*(3), 295–303. https://doi.org/10.1016/j.meatsci.2007.03.016

Lusk, J. L. (2018). Consumer preferences for and beliefs about slow growth chicken. *Poultry Science*, *97*(12), 4159–4166. https://doi.org/10.3382/ps/pey301

Magdelaine, P., Spiess, M. P., & Valceschini, E. (2008). Poultry meat consumption trends in Europe. *World's Poultry Science Journal*, *64*(1), 53–63.

Malila, Y., U-Chupaj, J., Srimarut, Y., Chaiwiwattrakul, P., Uengwetwanit, T., Arayamethakorn, S., . . . Visessanguan, W. (2018). Monitoring of white striping and wooden breast cases and impacts on quality of breast meat collected from commercial broilers (*Gallus gallus*). *Asian-Australasian Journal of Animal Science*, *31*(11), 1807–1817. https://doi.org/10.5713/ajas.18.0355.

Maxwell, A. D., Bowker, B. C., Zhuang, H., Chatterjee, D., & Adhikari, K. (2018). Descriptive sensory analysis of marinated and non-marinated wooden breast fillet portions. *Poultry Science*, *97*(8), 2971–2978. https://doi.org/10.3382/ps/pey145.

Meloche, K. J., Fancher, B. I., Emmerson, D. A., Bilgili, S. F., & Dozier III, W. A. (2018a). Effects of quantitative nutrient allocation on myopathies of the *Pectoralis major* muscles in broiler chickens at 32, 43, and 50 days of age. *Poultry Science*, *97*(5), 1786–1793. https://doi.org/10.3382/ps/pex453

Meloche, K. J., Fancher, B. I., Emmerson, D. A., Bilgili, S. F., & Dozier, W. A. (2018b). Effects of reduced digestible lysine density on myopathies of the Pectoralis major muscles in broiler chickens at 48 and 62 days of age. *Poultry Science*, *97*(9), 3311–3324. https://doi.org/10.3382/ps/pey171

Meloche, K. J., Fancher, B. I., Emmerson, D. A., Bilgili, S. F., & Dozier III, W. A. (2018c). Effects of reduced dietary energy and amino acid density on Pectoralis major myopathies in broiler chickens at 36 and 49 days of age1. *Poultry Science*, *97*(5), 1794–1807. https://doi.orghttps://doi.org/10.3382/ps/pex454

Meloche, K. J., Dozier III, W. A., Brandebourg, T. D., & Starkey J. D. (2018d). Skeletal muscle growth characteristics and myogenic stem cell activity in broiler chickens affected by wooden breast. *Poultry Science*, *97*(12), 4401–4414. https://doi.org/10.3382/ps/pey287.

Mudalal, S., Babini, E., Cavani, C., & Petracci, M. (2014). Quantity and functionality of protein fractions in chicken breast fillets affected by white striping. *Poultry Science*, *93*(8), 2108–2116. https://doi.org/10.3382/ps.2014-03911

Mudalal, S., Lorenzi, M., Soglia, F., Cavani, C., & Petracci, M. (2015). Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. *Animal*, *9*(4), 728–734. https://doi.org/10.1017/S175173111400295X

Mutryn, M. F., Brannick, E. M., Fu, W., Lee, W. R., & Abasht, B. (2015). Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing. *BMC Genomics*, *16*(1), 399. https://doi.org/10.1186/s12864-015-1623-0

Pampouille, E., Berri, C., Boitard, S., Hennequet-Antier, C., Beauclercq, S. A., Godet, E., . . . Le Bihan-Duval, E. (2018). Mapping QTL for white striping in relation to breast muscle yield and meat quality traits in broiler chickens. *BMC genomics*, *19*(1), 202. https://doi.org/10.1186/s12864-018-4598-9

Papah, M. B., Brannick, E. M., Schmidt, C. J., & Abasht, B. (2017). Evidence and role of phlebitis and lipid infiltration in the onset and pathogenesis of Wooden Breast Disease in modern broiler chickens. *Avian Pathology*, *46*(6), 623–643. https://doi.orghttps://doi.org/10.1080/03079457.2017.1339346

Papah, M. B., Brannick, E. M., Schmidt, C. J., & Abasht, B. (2018). Gene expression profiling of the early pathogenesis of wooden breast disease in commercial broiler chickens using RNA-sequencing. *Plos One*, *13*(12), e0207346. https://doi.org/10.1371/journal.pone.0207346

Papinaho, P. A., Ruusunen, M. H., Suuronen, T., & Fletcher, D. L. (1996). Relationship between muscle biochemical and meat quality properties of early deboned broiler breasts. *Journal of Applied Poultry Research*, *5*(2), 126–133. https://doi.org/10.1093/japr/5.2.126

Pearce, K. L., Rosenvold, K., Andersen, H. J., & Hopkins, D. L. (2011). Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes - A review. *Meat Science*, *89*(2), 111–124. https://doi.orghttps://doi.org/10.1016/j.meatsci.2011.04.007

Petracci, M., Mudalal, S., Bonfiglio, A., & Cavani, C. (2013). Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. *Poultry Science*, *92*(6), 1670–1675. https://doi.org/10.3382/ps.2012-03001

Petracci, M., Mudalal, S., Babini, E., & Cavani, C. (2014). Effect of White Striping on chemical composition and nutritional value of chicken breast meat. *Italian Journal of Animal Science*, *13*(1), 179–183. https://doi.org/10.4081/ijas.2014.3138

Petracci, M., Mudalal, S., Soglia, F., & Cavani, C. (2015). Meat quality in fast-growing broiler chickens. *World's Poultry Science Journal*, *71*(2), 363–374. https://doi.org/10.1017/S0043933915000367

Petracci, M., Soglia, F., & Berri, C. (2017). Muscle metabolism and meat quality abnormalities. In M. Petracci & C. Berri (Eds.), *Poultry quality evaluation. Quality attributes and consumer values* (pp. 51–75). Duxford, UK: Woodhead Publishing.

Radaelli, G., Piccirillo, A., Birolo, M., Bertotto, D., Gratta, F., Ballarin, C. Vascellari, . . . Trocino, A. (2017). Effect of age on the occurrence of muscle fiber degeneration associated with myopathies in broiler chickens submitted to feed restriction. *Poultry Science*, *96*, 309–319. https://doi.org/10.3382/ps/pew270

Russo, E., Drigo, M., Longoni, C., Pezzotti, R., Fasoli, P., & Recordati, C. (2015). Evaluation of White Striping prevalence and predisposing factors in broilers at slaughter. *Poultry Science*, *94*(8), 1843–1848. https://doi.org/10.3382/ps/pev172

Sachs, N. J., Hampton, A. R., Foster, K. K., Pechanec, M. Y., Henderson, J. D., King, A. J., & Mienaltowski, M. J. (2018). The effects of an alternative diet regimen with natural methionine ingredients on white striping breast myopathy in broiler chickens. *Poultry Science*, Advance online publication. https://doi.org/10.3382/ps/pey327.

Schilling, M. W., Suman, S. P., Zhang, X., Ciaramella, M. A., Allen, P. J., Nair, M. N., . . . Cai, K. (2017). Proteomic approach to characterize biochemistry of meat quality defects. *Meat Science*, *132*, 131–138. https://doi.org/10.1016/j.meatsci.2017.04.018

Sihvo, H. K., Immonen, K., & Puolanne, E. (2014). Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. *Veterinary Pathology*, *51*(3), 619–623. https://doi.org/10.1177/0300985813497488

Sihvo, H. K., Lindén, J., Airas, N., Immonen, K., Valaja, J., & Puolanne, E. (2017). Wooden breast myodegeneration of pectoralis major muscle over the growth period in broilers. *Veterinary Pathology*, *54*(1), 119–128. https://doi.org/10.1177/0300985816658099

Sihvo, H. K., Airas, N., Lindén, J., & Puolanne, E. (2018). Pectoral vessel density and early ultrastructural changes in broiler chicken wooden breast myopathy. *Journal of Comparative Pathology*, *161*(5), 1–10. https://doi.org/10.1016/j.jcpa.2018.04.002

Sirri, F., Maiorano, G., Tavaniello, S., Chen, J., Petracci, M., & Meluzzi, A. (2016). Effect of different levels of dietary zinc, manganese, and copper from organic or inorganic sources on performance, bacterial chondronecrosis, intramuscular collagen characteristics, and occurrence of meat quality defects of broiler chickens. *Poultry Science*, *95*(8), 1813–1824. https://doi.org/10.3382/ps/pew064

Soglia, F., Mudalal, S., Babini, E., Di Nunzio, M., Mazzoni, M., Sirri, F., . . . Petracci, M. (2016a). Histology, composition, and quality traits of chicken Pectoralis major muscle affected by wooden breast abnormality. *Poultry Science*, *95*(3), 651–659. https://doi.orghttps://doi.org/10.3382/ps/pev353

Soglia, F., Laghi, L., Canonico, L., Cavani, C., & Petracci, M. (2016b). Functional property issues in broiler breast meat related to emerging muscle abnormalities. *Food Research International*, *89*, 1071–1076. https://doi.org/10.1016/j.foodres.2016.04.042

Soglia, F., Gao, J., Mazzoni, M., Puolanne, E., Cavani, C., Petracci, M., & Ertbjerg, P. (2017). Superficial and deep changes of histology, texture and particle size distribution in broiler wooden breast muscle during refrigerated storage. *Poultry Science*, *96*(9), 3465–3472. https://doi.org/10.3382/ps/pex115

Soglia, F., Baldi, G., Laghi, L., Mudalal, S., Cavani, C., & Petracci, M. (2018b). Effect of White Striping on turkey breast meat quality. *Animal*, *12*(10), 2198–2204. https://doi.org/10.1017/S1751731117003469

Soglia, F., Mazzoni, M., & Petracci, M. (2019). Current growth-related breast meat abnormalities in broilers. *Avian Pathology*, *48*(1), 1–3. https://doi.org/10.1080/03079457.2018.1508821

Soglia, F., Zeng, Z., Gao, J., Puolanne, E., Cavani, C., Petracci, M., & Ertbjerg, P. (2018a). Evolution of proteolytic indicators during storage of broiler wooden breast meat. *Poultry Science*, *97*(4), 1448–1455. https://doi.orghttps://doi.org/10.3382/ps/pex398.

Soladoye O. P., Juarez M. L., Aalhus J. L., Shand P., & Estevez M. (2015). Protein oxidation in processed meat: Mechanisms and potential implications on human health. *Comprehensive Reviews in Food Science and Food Safety*, *14*, 106–122. https://doi.org/10.1111/1541-4337.12127

Straadt, I. K., Rasmussen, M., Andersen, H. J., & Bertram, H. C. (2007). Aging-induced changes in microstructure and water distribution in fresh and cooked pork in relation to water-holding capacity and cooking loss—A combined confocal laser scanning microscopy (CLSM) and low-field nuclear magnetic resonance relaxation study. *Meat Science*, *75*(4), 687–695. https://doi.org/10.1016/j.meatsci.2006.09.019

Sun, X., Koltes, D. A., Coon, C. N., Chen, K., & Owens, C. M. (2018). Instrumental compression force and meat attribute changes in woody broiler breast fillets during short-term storage. *Poultry Science*, *97*(7), 2600–2606. https://doi.org/10.3382/ps/pey107

Tallentire, C. W., Leinonen, I., & Kyriazakis, I. (2018). Artificial selection for improved energy efficiency is reaching its limits in broiler chickens. *Scientific Reports*, *8*(1), 1168. https://doi.org/10.1038/s41598-018-19231-2

Tasoniero, G., Cullere, M., Cecchinato, M., Puolanne, E., & Dalle Zotte, A. (2016). Technological quality, mineral profile, and sensory attributes of broiler chicken breasts affected by White Striping and Wooden Breast myopathies. *Poultry Science*, *95*(11), 2707–2714. https://doi.org/10.3382/ps/pew215

Tasoniero, G., Bertram, H.-C., Young, J.-F., Dalle Zotte, A., & Puolanne, E. (2017). Relationship between hardness and myowater properties in Wooden Breast affected chicken meat: A nuclear magnetic resonance study. *LWT - Food Science and Technology*, *86*, 20–24. https://doi.org/10.1016/j.lwt.2017.07.032

The Independent (2017). *Why you should stop eating chicken breast with white stripes*. Retrieved from https://www.independent.co.uk/life-style/health-and-families/chicken-breasts-white-stripes-avoid-poultry-factory-farms-fat-a7569656.html (last access 21/09/2018).

The Sun (2017). *This is what the white stripes in your chicken really are . . . and why you need to look out for them*. Retrieved from https://www.thesun.co.uk/fabulous/food/5241182/white-stripes-chicken/ (last access 21/09/2018).

Tijare, V. V., Yang, F. L., Kuttappan, V. A., Alvarado, C. Z., Coon, C. N., & Owens, C. M. (2016). Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. *Poultry Science*, *95*(9), 2167–2173. https://doi.org/10.3382/ps/pew129

Traffano-Schiffo, M. V., Castro-Giraldez, M., Colom, R. J., & Fito, P. J. (2017). Development of a spectrophotometric system to detect white striping physiopathy in whole chicken carcasses. *Sensors*, *17*, 1–14. https://doi.org/10.3390/s17051024

Trocino, A., Piccirillo, A., Birolo, M., Radaelli, G., Bertotto, D., Filiou, E., . . . Xiccato, G. (2015). Effect of genotype, gender and feed restriction on growth, meat quality and the occurrence of white striping and wooden breast in broiler chickens. *Poultry Science*, *94*(12), 2996–3004. https://doi.org/10.3382/ps/pev296

Utrera, M., & Estévez, M. (2012). Oxidation of myofibrillar proteins and impaired functionality: Underlying mechanisms of the carbonylation pathway. *Journal of Agricultural and Food Chemistry*, *60*, 8002–8011. https://doi.org/10.1021/jf302111j

Wideman, N., O'Bryan, C. A., & Crandall, P. G. (2016). Factors affecting poultry meat colour and consumer preferences—A review. *World's Poultry Science Journal*, *72*(2), 353–366.

Velleman, S. G., Clark, D. L., & Tonniges, J. R. (2017). Fibrillar collagen organization associated with broiler wooden breast fibrotic myopathy. *Avian Diseases*, *61*(4), 481–490. https://doi.org/10.1637/11738-080217-Reg.1

Vignale, K., Caldas, J. V., England, J. A., Boonsinchai, N., Magnuson, A., Pollock, E. D., ... Coon, C. N. (2017). Effect of white striping myopathy on breast muscle (*Pectoralis major*) protein turnover and gene expression in broilers. *Poultry science*, *96*, 886–893. https://doi.org/10.3382/ps/pew315

Wold, J. P., Veiseth-Kent, E., Høst, V., & Løvland, A. (2017). Rapid on-line detection and grading of wooden breast myopathy in chicken fillets by near-infrared spectroscopy. *PloS One*, *12*(3), e0173384. https://doi.org/10.1371/journal.pone.0173384

Wold, J. P., Måge, I., Løvland, A., Sanden, K. W., & Ofstad, R. (2018). Near-infrared spectroscopy detects woody breast syndrome in chicken fillets by the markers protein content and degree of water binding. *Poultry Science*, Advance online publication. https://doi.org/10.3382/ps/pey351.

Xing, T., Zhao, X., Han, M., Cai, L., Deng, S., Zhou, G., & Xu, X. (2017a). A comparative study of functional properties of normal and wooden breast broiler chicken meat with NaCl addition. *Poultry Science*, *96*(9), 3473–3481. https://doi.org/10.3382/ps/pex116

Xing, T., Zhao, X., Cai, L., Zhou, G., & Xu, X. (2017b). Effect of salt content on gelation of normal and wooden breast myopathy chicken pectoralis major meat batters. *International Journal of Food Science and Technology*, *52*, 2068–2077. https://doi.org/10.1111/ijfs.13485

Yoon, S.-C., Bowker, B. C., & Zhuang, H. (2017). Toward a fusion of optical coherence tomography and hyperspectral imaging for poultry meat quality assessment. *IS and T International Symposium on Electronic Imaging Science and Technology*, https://doi.org/10.2352/ISSN.2470-1173.2016.14.IPMVA-380

Zambonelli, P., Zappaterra, M., Soglia, F., Petracci, M., Sirri, F., Cavani, C., & Davoli, R. (2016). Detection of differentially expressed genes in broiler Pectoralis major muscle affected by White Striping—Wooden Breast myopathies. *Poultry Science*, *95*(12), 2171–2785. https://doi.org/10.3382/ps/pew268

Zampiga, M., Soglia, F., Petracci, M., Meluzzi, A., & Sirri, F. (2018). Effect of different arginine to lysine ratios in broiler chicken diets on the occurrence of breast myopathies. *Poultry Science*, *9*, 79. https://doi.org/10.3382/ps/pey284

Zimermann F. C., Fallavena, L. C. B., Salle, C. T. P., Moraes, H. L. S., Soncini, R. A., Barreta, M. H., & Nascimento, V. P. (2012). Downgrading of heavy broiler chicken carcasses due to myodegeneration of the anterior latissimus dorsi: Pathologic and epidemiologic studies. *Avian Diseases*, *56*, 418–421.

Zhuang, H., & Bowker, B. (2018). The wooden breast condition results in surface discoloration of cooked broiler pectoralis major. *Poultry Science*, *97*(12), 4458–4461. https://doi.org/10.3382/ps/pey284

3 MATERIAL E MÉTODOS

3.1 DELINEAMENTO EXPERIMENTAL

As atividades desenvolvidas na tese foram executas em abatedouro frigorífico com Inspeção Federal (Brasil), na Universidade Federal da Paraíba (UFPB, Brasil) e na Universidade de Extremadura (UEx, Espanha), conforme apresentado na Figura 1.

O primeiro estudo foi desenvolvido na linha de produção do abatedouro comercial localizado no Estado da Paraíba, região Nordeste do Brasil e nos laboratórios pertencentes ao Departamento de Engenharia de Alimentos (DEA) do *Campus I* da UFPB (João Pessoa, Paraíba, Brasil).

O segundo estudo foi executado nos laboratórios do Departamento de Tecnologia de Alimentos da UEx (Cáceres, Espanha), através do Programa de Doutorado Sanduíche no Exterior (SWE/CNPq), processo nº 208241/2017-5, no período de dez meses, de março de 2018 a dezembro de 2018.

O experimento realizado no Brasil envolveu o estudo da ocorrência das miopatias *White Striping* (WS) e *Wooden Breast* (WB), isoladas ou combinadas, em aves abatidas com diferentes faixas etárias, através da aplicação de técnica clássica de identificação, baseada no aspecto visual e palpação do músculo, com posterior avaliação do efeito dessas miopatias na qualidade físico-química de peitos de frango. Além disso, no intuito de avaliar a viabilidade de uma técnica rápida, barata, não destrutiva e que pode ser incorporada na linha de processo dentro do abatedouro, foi usada espectroscopia do infravermelho próximo (NIRS) associado à análise multivariada para distinguir músculos afetados pelas miopatias WS e WB em linha de produção.

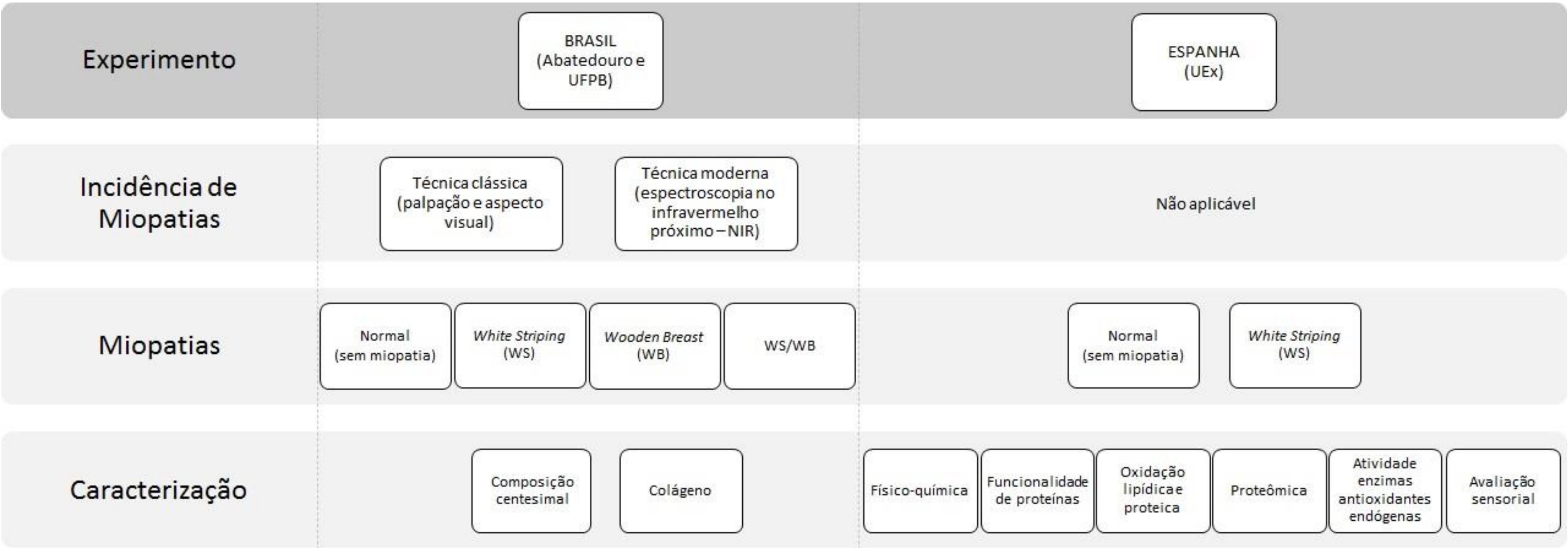
No estudo de ocorrência das miopatias WS e WB verificou-se cerca de 10 % do volume de abate diário do abatedouro. Para tanto, analisou-se a ocorrência de miopatias WS e WB no músculo *Pectoralis major* provenientes de aves abatidas com quatro diferentes faixas etárias. As faixas de idade de abate resultaram do planejamento de abate da empresa, o qual ocorreu durante os estudos de ocorrência. No estudo envolvendo o uso da espectroscopia NIR foram avaliados 1028 músculos *P. major* de aves abatidas com as mesmas quatro faixas de idade de abate. Este estudo foi realizado simultaneamente com a técnica visual e de palpação.

Das amostras analisadas por aspecto visual, palpação e NIR, foram selecionados 32 peitos sem pele de aves abatidas com 6-7 semanas (por representarem a maior parcela de volume de abate), referente a 4 tratamentos (Normal, WS, WB, WS/WB) e 8 repetições cada.

As amostras foram encaminhadas aos laboratórios do DEA/UFPB para caracterização físico-química.

O estudo executado na UEx/Espanha teve por objetivo aprofundar os conhecimentos sobre o efeito das miopatias WS na qualidade de peitos de frango, enfatizando-se os parâmetros oxidativos, além da qualidade sensorial de peitos de frango acometidos com a anomalia WS. Para a avalição do efeito das miopatias na qualidade oxidativa de carne WS, foram avaliados 3 tratamentos: Normal (n=10), WS-grau moderado (n=10) e WS-grau severo (n=8), totalizando-se 28 amostras. Para a avaliação sensorial de aceitabilidade e intenção de compra foram utilizados 3 tratamentos, a saber, Normal (n=4), WS-moderado (n=4) e WS-severo (n=4), e para a avaliação das emoções evocados durante o consumo dessas carnes foram utilizados 2 tratamentos: Normal (n=10) e WS-severo (n=10).

Figura 1 - Esquema dos experimentos realizados no Brasil e na Espanha



WS/WB: peitos que apresentam simultaneamente as miopatias WS e WB.

3.2 EXPERIMENTOS REALIZADOS NO BRASIL

3.2.1 Ocorrência de miopatias

O estudo da ocorrência das miopatias WS e WB em abatedouro localizado no Estado da Paraíba, região Nordeste do Brasil, foi realizado no período de agosto a dezembro de 2017. Um total de 8.959 peitos de aves da linhagem *Cobb* foram analisados sendo estes distribuídas em quatro faixas de idades de abate: 4-5 semanas (frango com peso vivo de 1,94 kg, n=1.200 aves), 6-7 semanas (frango com peso vivo de 3,18 kg, n= 6.919 aves), 8-9 semanas (frango com peso vivo de 4,70 kg, n= 600 aves) e 65 semanas de vida (galinha matriz com peso vivo de 4,08 kg, n= 240 aves).

O volume de abate diário da planta comercial é de aproximadamente 85.000 aves, das quais, 13% correspondem a frangos com 4-5 semanas de vida, 77% a frangos com 6-7 semanas, 7% a frangos com 8-9 semanas e 3% de galinha matriz com 65 semanas.

A pesquisa, por envolver o uso de animais, foi submetida ao Comitê de Ética no Uso de Animais – CEUA/UFPB (protocolo nº 031/2017). O parecer favorável está disponível no ANEXO A.

3.2.1.1 Classificação das miopatias

Após o abate, seguindo o fluxograma clássico constituído de: pendura, atordoamento por eletronarcose, sangria, escalda, depenagem, evisceração, inspeção, pré-resfriamento de carcaça e corte, os peitos foram classificados na linha de processamento em normais ou com miopatias WS e WB (isoladas ou combinadas), tomando-se por base o método tradicional de identificação visual (aspecto visual) e apalpação (consistência de palpação) do músculo *Pectoralis major*, de acordo com o Quadro 1.

Quadro 1 - Classificação das miopatias

Classificação	Grau	Tratamento	Descrição
Normal	-	N	Peito sem áreas endurecidas ou estrias brancas aparentes na superfície do músculo
White Striping (WS)	Moderado	WS-M	Peitos afetados com estrias brancas de espessura inferior a 1mm, com área ocupada > ou < 1/2 da superfície do músculo
	Severo	WS-S	Peitos afetados com estrias brancas de espessura superior a 1mm, com área ocupada > ou < 1/2 da superfície do músculo
Wooden Breast (WB)	Suave	WB-I	Peitos afetados com dureza focal na região cranial, com/sem pontos de hemorragia na superfície
	Moderado	WB-M	Peitos afetados com dureza moderada e extensiva por toda superfície, com/sem pontos de hemorragia na superfície
	Severo	WB-S	Peitos afetados com dureza extrema e extensiva por toda superfície, com/sem pontos de hemorragia na superfície
WS/WB	WB-I e WS-M	WS/WB-1	Peitos afetados por dureza focal na região cranial com estrias brancas de espessura inferior a 1mm. Com/sem pontos de hemorragia na superfície
	WB-I e WS-S	WS/WB-2	Peitos afetados por dureza focal na região cranial com estrias brancas de espessura superior a 1mm. Com/sem pontos de hemorragia na superfície
	WB-M e WS-M	WS/WB-3	Peitos afetados por dureza moderada e extensiva com estrias brancas de espessura inferior a 1mm. Com/sem pontos de hemorragia na superfície
	WB-M e WS-S	WS/WB-4	Peitos afetados por dureza moderada e extensiva com estrias brancas de espessura superior a 1mm. Com/sem pontos de hemorragia na superfície
	WB-S e WS-M	WS/WB-5	Peitos afetados por dureza extrema e difusa com estrias brancas de espessura inferior a 1mm. Com/sem pontos de hemorragia na superfície
	WB-S e WS-S	WS/WB-6	Peitos afetados por dureza extrema e difusa com estrias brancas de espessura superior a 1mm. Com/sem pontos de hemorragia na superfície

3.2.1.2 Caracterização físico-química dos peitos N, WS, WB, WS/WB

Os peitos após a classificação por palpação, referentes a aves de idade de abate de 6-7 semanas foram analisados em relação a sua composição físico-química. Após coleta no setor de cortes (cerca de 2 horas após abate), os peitos (T ≤ 7 °C) foram embalados individualmente em sacos de polietileno do tipo *Zip Lock*, resfriados e transportados ao Laboratório sob refrigeração (T ≤ 4 °C).

O teor de proteínas foi estimado por Kjeldahl (AOAC, 2000) (método nº 981.10) utilizando o fator de conversão de 6,25. O teor de umidade foi determinado em estufa a 105°C por 24 h (AOAC, 2000) (método nº 950.46). O teor de colágeno foi determinado por método colorimétrico baseado na quantidade de hidroxiprolina presente na amostra (AOAC, 2000) (método nº 990.26), considerando o fator de conversão de 8,0. O conteúdo total de lipídios foi avaliado de acordo com método proposto por Folch, Less e Stanley (1957).

3.2.2 Espectroscopia de infravermelho próximo (NIRS)

3.2.3.1 Medições de espectros NIR

O espectrofotômetro portátil (DLP NIRscan Nano GUI v2.1.0, Texas Instruments) coletou absorbâncias em 228 comprimentos de ondas entre 900 e 1700 nm com resolução espectral de 10 nm de intervalo e 10 varreduras por comprimento de onda. O detector foi posicionado em contato direto com os peitos. O procedimento levou cerca de 5 s por amostra. Foram realizadas leituras na região cranial, superfície externa, no peito sem pele. As leituras foram realizadas na mesma região para todas as amostras. A temperatura do ambiente foi controlada em 12 ± 2ºC durante todo o processo de aquisição espectral.

3.2.3.2 Processamento dos dados espectrais

O espectro NIR foi usado para construir modelos de classificação usando o Algoritmo das Projeções Sucessivas - Análise Discriminante Linear (SPA-LDA) e Modelagem Suave Independente por Analogia de Classe (SIMCA). Os dados brutos foram pré-processados através do alisamento Savitzky-Golay, 1ª e 2ª derivadas Savitzky-Golay, Variável Normal Padrão (SNV) e Correção de Espalhamento Multiplicativo (MSC). A análise de componentes principais (PCA) foi usada para discriminação da amostra. Gráficos de escores, resíduos e T^2 de Hotelling foram empregados na detecção e eliminação de outliers usando PCA robusta (RPCA). Os dados pré-processados foram separados em conjuntos de treinamento (60% das amostras), validação (20% das amostras) e teste (20% das amostras) usando o algoritmo de Kennard-Stone (KS) (SILVA et al., 2013). O algoritmo KS foi aplicado separadamente à cada classe. O tratamento de dados quimiométricos foi implementado com o software Matlab (R2010a, versão 7.10.0.499, The Mathworks, Inc., Natick, MA) equipado com um pacote estatístico multivariado.

3.3 EXPERIMENTOS REALIZADOS NA UEX-ESPANHA

Os experimentos desenvolvidos na Espanha que enfatizaram o entendimento do efeito da miopatia WS sobre os aspectos oxidativos e sua qualidade sensorial utilizaram peitos de frango resfriados adquiridos em quatro supermercados localizados na cidade de Cáceres, Espanha.

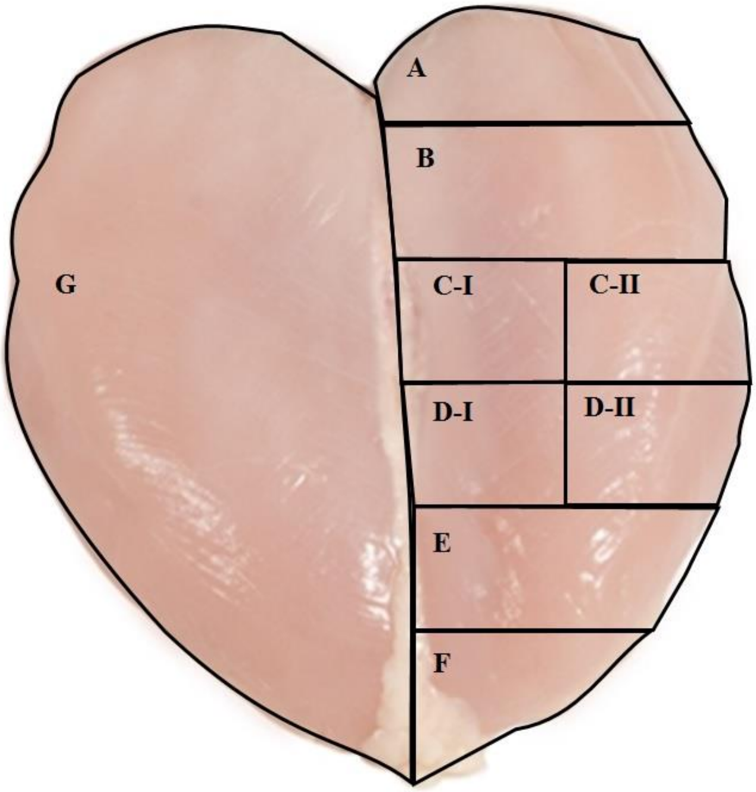
Três tratamentos foram selecionados para os experimentos de oxidação e qualidade sensorial de peitos WS, baseado nos critérios descritos por Kuttappan et al. (2012a): N (Normal, não apresenta estrias brancas na superfície do músculo), WS-M (Estriado Moderado, exibe estrias brancas com espessura < 1 mm, em toda superfície do músculo) e WS-S (Estriado Severo, exibe estrias brancas com espessura > 1mm, em toda extensão do músculo).

3.3.1 Aspectos oxidativos de peitos N e WS

No estudo envolvendo os aspectos oxidativos de peitos WS, as amostras foram fracionadas, conforme a Figura 2. Nas porções A e B foram realizadas as análises de caracterização físico-química (CRA, TPA, umidade, proteína, lipídio e colágeno), imediatamente após o fracionamento do peito. As porções C, D, E e F foram congeladas a -80 °C para estudos posteriores de avaliação do dano oxidativo aos lipídios (TBARS) e proteínas (alisina, base de Schiff, pontes dissulfeto e tióis livres) e atividade *in vitro* de enzimas antioxidantes (catalase, superóxido disminutase e glutationa peroxidase). A porção

G foi submetida a análise de proteômica para discriminação das proteínas sarcoplasmáticas após 24 horas do fracionamento do peito.

Figura 2 - Esquema do fracionamento do peito



A: Caracterização físico-química, capacidade de retenção de água; B: Perfil de textura instrumental (TPA); C-I: TBARS (amostra crua); C-II: TBARS (amostra cozida); D-I: Determinação de alisina, bases de Shiff, tióis livres e pontes dissulfeto (amostras cruas); D-II: Determinação de alisina, bases de Schiff, tióis livres e pontes dissulfeto (amostras cozidas); E: Atividade enzimas antioxidantes (CAT, GSH-Px, SOD); F: Porção reserva; G: Proteômica (futuro trabalho).

3.3.1.1 Caracterização físico-química

A determinação do teor de proteína, lipídios, umidade e colágeno foi realizada conforme descrito no item 3.2.1.2, na porção A das amostras.

Os parâmetros de cor e pH foram determinados antes do fracionamento das amostras. A cor foi determinada através da medição das coordenadas L* (*lightness*), a* (*redness*) e b* (*yellowness*), por meio de colorímetro digital (CR400, Konica Minolta Sensing Inc., Osaka, Japão). Para a leitura foram fixadas as seguintes condições: iluminante C, ângulo de visão 8°, ângulo padrão do observador 10°, especular incluída, conforme especificações da *Comission Internationale de L'éclairage* (CIE, 1986). O instrumento, antes da realização das leituras, foi calibrado em placa de cerâmica branca (Iluminante C: Y= 92,84 X=0,3136, y=0,3201). A cor foi avaliada na superfície interna em três diferentes regiões do peito: proximal, distal e no ponto médio entre essas duas extremidades (CARVALHO et al, 2017).

O pH foi determinado através da inserção de eletrodo na região cranial do peito utilizando o pHmetro de contato (HI 99163, Hanna Instruments, Woonsocket, Rhode Island, USA), de acordo com a metodologia proposta por Boulianne e King (1998).

A dureza foi determinada através da análise de perfil de textura instrumental (TPA) na porção B dos peitos de frango crus. As amostras foram cortadas em paralelepípedos de 25 x 25 x 10 mm com faca de lâmina afiada e auxílio de uma régua, e analisadas em dois ciclos de compressão em texturômetro (TA.XTplusC, Stable Microsystems, Godalming, Surrey, Reino Unido). As condições de teste foram: compressão de 50% da altura original, velocidade pré-teste e de teste 50 mm/min, *probe* de compressão de 5 cm de diâmetro (P/50), conforme descrito por Chatterjee et al., 2016, com adaptações.

A capacidade de retenção de água (CRA) foi determinada por centrifugação, conforme método descrito por Carvalho et al. (2017), com algumas mudanças. Foram pesadas 6 g de amostra crua (porção A) triturada em tubo de 50 mL e a adicionado de 10 mL de solução de NaCl 0.6 M, o tubo foi vigorosamente agitado por cerca de 15 s. Posteriormente aquecidos a 80 °C por 30 min em banho de água. Posteriormente, as amostras foram incubadas a 4 ºC por 30 min e centrifugados por 15 min a 3.500 rpm a 4 °C. Em seguida, o sobrenadante foi descartado e medido o peso do tudo com o precipitado. CRA foi medida como percentual de perda de água durante o cozimento. E calculado conforme a Equação 1.

$$CRA\ (\%) = 100 - \left[\left(\frac{Peso\ inicial\ da\ amostra - Peso\ final\ da\ amostra}{Peso\ inicial\ da\ amostra}\right) \times 100\right] \qquad \text{Equação 1}$$

3.3.1.2 Avaliação da oxidação lipídica

3.3.1.2.1 Determinação do índice de TBARS

As substâncias reativas ao ácido tiobarbitúrico foram determinadas conforme descrito por Ganhão, Estévez e Morcuende (2011). A curva padrão foi preparada usando uma solução mãe de 1,1,3,3 tetraetoxipropano (TEP) em ácido perclórico a 3,86% ($0,233.10^{-3}$ g/mL), ao qual variou de $1,9.10^{-3}$ a $3,8.10^{-4}$ mg malonaldeído (MDA)/mL. Foram pesados cerca de 4 g de amostras, referentes as porções C-I e C-II, conforme apresentado na Figura 2. Após pesagem das amostras, foi adicionado 6 mL e 8,9 mL de ácido perclórico a 3,86%. A mistura foi homogeneizada em turrax (6.000 rpm por 30 s) com tubo de falcon introduzido dentro de um bécker contendo gelo para evitar o aquecimento da amostra. O material foi filtrado em papel de filtro duplo, e centrifugado a 3.500 rpm por 3 min, obtendo o extrato da amostra. Foram tomados 2 mL do extrato preparado e adicionado a tubo rosqueado, acrescentou-se 2 mL de TBA 0,02 M. Para o branco, foi utilizado 2 mL de ácido perclórico a 3,86% e 2 mL de TBA 0,02 M. Os tubos foram aquecidos em banho de água a 100 °C por 30 min, resfriados e centrifugados a 3.500 rpm por 2 min. Posteriormente, as amostras foram lidas em espectrofotômetro a 532 nm. TBARS foram expressas como mg de $MDA.kg^{-1}$ de amostra.

3.3.1.3 Avaliação da oxidação proteica

3.3.1.3.1 Obtenção de extrato de proteínas miofibrilares e sarcoplasmáticas

Os extratos de proteínas sarcoplasmáticas (PS) e miofibrilares (PM) foram obtidos a partir das frações D-I (amostras cruas) e D-II (amostras cozidas), conforme metodologia descrita por Zhu et al. (2011), com modificações. Para extração de PS, foi pesado cerca de 2,5 g para carnes WS. Em seguida, as amostras foram homogeneizadas em 10 mL de tampão contendo fosfato de sódio 10 mM, NaCl 0,1 N, $MgCl_2$ 2 mM e EGTA 1 mM, pH 7.0, com turrax a 9000 rpm por 30 s. O homogeneizado foi centrifugado a 2000 rpm por 15 min a 4 °C, o sobrenadante foi reservado e o precipitado foi ressuspendido e homogeneizado em 10 mL do tampão, e novamente centrifugado sob igual condição. O sobrenadante foi combinado com o anterior e armazenado sob refrigeração (extrato PS). O precipitado foi ressuspenso em 10 mL de NaCl 0,1 N, homogeneizado e filtrado em gaze (remoção do tecido conectivo). O filtrado, foi centrifugado a 2000 rpm por 15 min a 4 °C, o sobrenadante foi descartado e o precipitado foi suspendido em tampão fosfato de sódio 10 mM e NaCl 0,6 N, pH 6.5 (extrato PM) e armazenado sob refrigeração.

3.3.1.3.2 Determinação de alisina

A determinação de alisina foi realizada conforme o método descrito por Utrera et al. (2011), com adaptações. Para esta determinação foram utilizados os extratos de proteínas miofibrilares obtidos a partir das frações D-I e D-II (ver item 3.3.4.2). Uma alíquota de 250 µL desses extratos foi transferido para um eppendorf de 2 mL, em seguida adicionado e 1,5 mL de TCA 5 % e homogeneizado em vórtex. A mistura foi centrifugada a 5.000 rpm por 5 min a 4°C. O sobrenadante foi descartado e as proteínas precipitadas foram adicionadas 0,5 mL de SDS 1% e DPTA 1 mM em tampão MES 0,25 M pH 6, e misturado em vórtex, em seguida foi acrescentado 0,5 mL ABA 50 mM em tampão MES 0,25 M pH 6 e 0,25 mL de $NaBH_3CN$ 100 mM em tampão MES 0,25 M pH 6. A mistura foi agitada em vórtex e foram incubadas em estufa a 37 °C por 90 min, com agitação a cada 30 min. Após a derivatização, acrescentou-se 0,25 mL de TCA a 50% para parar o processo, e agitou-se novamente os tubos, abrindo entre uma agitação e outra a tampa para a saída do gás formado. Após esta etapa, a mistura foi centrifugada a 5000 rpm por 10 min a 4 °C e o sobrenadante descarto. O precipitado foi lavado com 0,75 mL de TCA a 5% e 0,75 mL de etanol:acetato de etila (1:1 v/v) e centrifugado a 5.000 rpm por 5 min a 4 °C e o sobrenadante eliminado, este procedimento foi repetido. Após a lavagem das amostras, foi adicionado ao precipitado 1 mL de HCl 6 M e armazenados durante 18 h em estufa a 110 °C. Após esse período, as amostras hidrolisadas foram secas em gás nitrogênio, reconstituídas com 200 µL de água Milli-Q e filtradas em filtro de seringa de fluoreto de polivinilideno (tamanho de poro 0,45 µm, Pall Corp., New York, USA). As amostras (1,0 µL) foram injetadas em sistema de cromatografia líquida (Shimadzu Prominence HPLC, Shimadzu Corp., Kyoto, Japan) acoplado a um detector de fluorescência (Shimadzu Corp., RF-10A XL, Kyoto, Japan), coluna C18-AR-II RP-HPLC (150 x 4,6 mm I.D., Cosmosil, Phenomenex, California, USA) e uma pré-coluna (10 x 4,6 mm) com o mesmo material. Foram utilizados como eluente a acetonitrila e o tampão acetato de sódio 50 mM, pH 5,4. O comprimento de onda de emissão foi lido a 283 nm e o de excitação a 350 nm. A separação cromatográfica ocorreu a fluxo constante de 1 mL/min, pressão de 7,0 MPa e temperatura da coluna a 30 ºC. A identificação dos semialdeídos derivatizados foi realizada por comparação dos tempos de retenção das amostras com um padrão injetado nas mesmas condições anteriormente descritas. O pico correspondente a alisina-ABA foi integrado manualmente e as áreas resultantes foram plotadas em uma curva padrão de ABA, a qual variou de 0,1 a 0,5 mM. Para estimar as quantidades de alisina-ABA, considerou-se que a fluorescência emitida por 1 mol de ABA é equivalente àquela emitida por 1 mol de compostos carbonílicos proteicos derivatizados. Os resultados foram expressos em nmol de alisina por mg de proteína.

3.3.1.3.3 Determinação de Bases de Schiff

A análise das bases fluorescentes de Schiff foi realizada utilizando espectroscopia de fluorescência, conforme descrito por Chelh, Gatellier e Santé-Lhoutellier (2007). Os homogenatos da amostra em tampão fosfato de sódio pH 6,0 com uréia 8M (1:10 p/v, porção D-I e D-II) foram obtidos usando um ultraturrax. Após diluição (1:20 v/v), as amostras foram transferidas para uma cubeta de quartzo de 4 mL com quatro paredes planas (101-QS 10 × 10 mm, Hellma Analytics, Müllheim, Alemanha). O espectro de emissão para a base Schiff foi registrado entre 400 nm e 500 nm de comprimento de onda com excitação definida em 350 nm (espectrômetro Perkin-Elmer LS 55 Luminescence, Beaconsfield, Reino Unido). As larguras de fenda de excitação e emissão foram fixadas em 10 nm e os dados foram coletados em 500 nm por minuto. Os resultados foram expressos como unidades de intensidade de fluorescência emitidas pelas estruturas de base de Schiff a 450 nm. Esses valores foram corrigidos de acordo com a concentração de proteínas de cada amostra, aplicando um fator de correção (Cf = Pt/Pp) em que Pt é a média total da quantidade de proteína de todas as amostras e Pp é o conteúdo de proteína em cada tipo de amostra.

3.3.1.3.4 Determinação de Tióis livres e pontes dissulfeto

Os tiós livres e pontes dissulfeto foram analisados conforme método proposto por Rysman et al. 2014, com modificações. Foi utilizado o extrato de proteínas miofibrilares referente as porções D-I e D-II, obtidos conforme item 3.3.3.1 (mesmo extrato utilizado para a determinação dos semialdeídos AAS e GGS). Uma alíquota de 3 mL do extrato foi precipitada com 2,6 mL de TCA a 10% em tubos de vidro rosqueados e após agitação em vórtex, os tubos foram centrifugados a 4.000 rpm por 4 mim a 4 °C. O sobrenadante foi removido e adicionou-se 3,25 mL de hidrocloreto de guanidina 6M em tampão Tris 100 mM, pH 8. Os tubos foram novamente agitados em vórtex e centrifugados a 4.000 rpm por 20 min a 4 °C, e o sobrenadante foi filtrado (papel de filtro qualitativo, 11 μm). Foi retirada uma alíquota de 250 µL do sobrenadante (amostra não reduzida) e reservada; os 3 mL restantes foram submetidos a redução por adição de 50 µL de 1-octanol e 100 µL de boro-hidreto de sódio a 30% em NaOH 1 M, seguido de incubação a 50 °C por 30 min. Após incubação, foi adicionado lentamente uma alíquota de 1,35 mL de HCl 6 M, e os tubos foram mantidos sob agitação por 10 min, sendo reservada uma alíquota de 250 µL (amostra reduzida). Os tióis livres e totais foram determinados com 4,4-ditiodipiridina (4-DPS) tanto para as amostras não reduzidas quanto para as reduzidas. Para isso, alíquotas de 250 µL das amostras reduzidas e não reduzidas foram misturadas 1 mL de cloridrato de guanidina (GuHCl) 6 M em tampão de ácido cítrico 1 M, pH 4.5, e em seguida, sendo lidas em espectrofotômetro a 324 nm (A_{pre}). Após leitura, foi adicionado 250 µL de 4-DPS, seguido de incubação a temperatura ambiente na ausência de luz por 30 min. Após esse período as amostras foram novamente lidas a 324 nm (A_{pos}). Uma mistura de 1,25 mL de GuHCl 6 M em tampão ácido cítrico 1 M pH 4,5, e 250 µL de 4-DPS 4 mM em HCl 12 mM foi utilizada como branco (A_{branco}). A concentração de tiol foi calculada com base numa curva padrão de 2,5 a 500 µM de L-cisteína em GuHCl 6 M em tampão ácido cítrico 1 M (pH 4,5). O teor de tióis livres e totais foram expressos como nmoles de tióis por mg de proteína, e o teor de pontes dissulfeto foi calculado como metade da diferença entre os tióis totais e os tióis livres.

3.3.1.4 Estudo *in vitro* da atividade de enzimas antioxidantes (CAT, GSH-Px, SOD)

3.3.1.4.1 Extração para determinação da atividade de enzimas antioxidantes

A extração foi realizada na carne de peito, seguindo o método descrito por Carvalho et al. (2017). As extrações foram realizadas duas vezes com uma réplica. O músculo (5 g) foi misturado com 35 mL de tampão fosfato resfriado (solvente de extração, pH 7,0, 50 mM; fosfato dissódico hepta-hidratado (Na_2HPO_4 x $7H_2O$) e KH_2PO_4). As amostras foram homogeneizadas com o auxílio de ultraturrax (12.000 rpm por 45 s). Após centrifugação (4500 g, 40 min, 4 °C), os sobrenadantes foram recuperados e filtrados em lã de vidro. Os extratos musculares foram utilizados para as medidas enzimáticas da catalase (CAT), glutationa peroxidase (GSH-Px) e superóxido dismutase (SOD).

3.3.1.4.2 Atividade da catalase (CAT)

A atividade da catalase foi medida de acordo com Carvalho et al. (2017), com pequenas modificações. Uma alíquota de 100 µL de extrato de carne de peito (mantida no banho de gelo) foi transferida para uma cubeta (1 cm de comprimento) e foram adicionados 2,90 mL de H_2O_2. Imediatamente, a absorbância foi monitorada a 240 nm por 180 segundos usando um espectrofotômetro (Shimadzu UV-1800, Japão). A atividade de CAT foi expressa em µmol × min^{-1} × g^{-1} (U/g). Uma unidade (U) de atividade de CAT foi definida como a quantidade de extrato necessária para decompor 1 µmol de H_2O_2 por minuto.

3.3.1.4.3 Atividade da glutationa peroxidase (GSH-Px)

A GSH-Px foi determinada na carne de peito usando o método descrito por Carvalho et al. (2017), com pequenas modificações. Uma alíquota de 600 µL dos extratos musculares foi misturada com 2,35 mL de solvente de reação contendo 1,13 mM de glutationa reduzida, 0,57 mM de EDTA, 1,13 mM de NaN3 e 1,7 unidades de glutationa redutase em 100 mL de tampão fosfato (pH 7,0, 50 mM; fosfato dissódico hepta-hidratado ($Na_2HPO_4.7H_2O$) e KH_2PO_4 em água MiliQ). Foram distribuídos 26 µL de solução de NADPH (17,3 mM) e 20 µL de solução de H_2O_2 em cubetas (1 cm de comprimento). A absorbância foi monitorada a 340 nm por 600 segundos usando um espectrofotômetro (Shimadzu UV-1800). O coeficiente de extinção de 6,220 µl µmol-1 cm-1 para NADPH a 340 nm e 25 ° C foi utilizado para o cálculo. A atividade de GSH-Px foi expressa como µmol de NADPH $µL^{-1}$ min^{-1} g^{-1} oxidado (U/g).

3.3.1.3.4 Atividade da superóxido dismutase (SOD)

A superóxido dismutase foi medida de acordo com o procedimento de Marklund e Marklund (1974) usando a inibição da auto-oxidação de pirogalol em um meio básico. A fração sobrenadante do homogenato muscular também foi usada para a determinação da atividade da enzima SOD; 50 µl de pirogalol (10 mM) foram adicionados a 2,9 ml de tampão Tris-cacodílico (pH = 8,2, 50 mM com ácido dietilenotriaminopentaacético, DTPA). A taxa de auto-oxidação do pirogalol na presença de 50 µl de extrato muscular foi comparada a um branco (com 50 µl de tampão) medindo o aumento da absorvância a 420 nm durante 360 segundos usando um espectrofotômetro (Shimadzu UV-1800). Uma unidade foi tomada como a atividade que inibiu a auto-oxidação do pirogalol em 50%.

3.3.1.5 Análise de Proteômica

3.3.1.5.1 Extração e quantificação de proteínas

As proteínas foram extraídas de amostras compradas no dia anterior a realização da análise e mantidas sobre refrigeração (4 °C). A análise foi realizada em peitos classificados como Normal e WS-severo, totalizando 10 peitos investigados (2 tratamentos e 5 repetições).

A extração da fração de proteínas sarcoplasmáticas foi extraída conforme Zhu et al. (2011), como algumas alterações. Foram pesados 20 g de carne (meio filé de peito triturado) em tudo de centrífuga de 250 mL e adicionado 80 mL do tampão de proteínas sarcoplasmáticas (fosfato de sódio 10 mM, NaCl 0,1 N, $MgCl_2$ 2 mM e EGTA 1 mM, pH 7.0), em seguida, foram homogeneizados em turrax (9.000 rpm por 30 segundos) e posteriormente centrifugado a 3000 rpm por 15 min. O sobrenadante foi reservado, e o precipitado foi ressuspendido em 80 mL do mesmo tampão, agitado em vórtex e centrifugado na mesma condição. Os sobrenadantes foram combinados, obtendo-se o extrato de proteínas sarcoplasmáticas. Em seguida, foi retirado uma alíquota de 50 µL do extrato PS e dilui-se em 950 µL do tampão PS (DC – diluição controle), e determinado o teor de proteínas por Bradford (Bradford, 1976) para cada amostra.

Posteriormente foi adicionado 250 µL de TCA 50% frio a DC (concentração final de TCA na solução de 10%), agitou-se em vórtex brevemente e incubou-se em gelo por 30 min na ausência de luz. As amostras foram centrifugadas a 12.000 rpm por 10 min a 4 °C e o sobrenadante foi descartado. Em seguida, foi adicionado 500 µL de acetona PA gelada (-20 °C) e o precipitado rompido sob agitação em vórtex, e depois, incubado a -20 °C por 1 hora no congelador. As amostras foram novamente centrifugadas (12.000 rpm, 10 min, 4 °C) e o sobrenadante descartado. Este procedimento se repetiu por mais 2 vezes. Os precipitados obtidos após as lavagens com acetona foram secos a temperatura ambiente e, imediatamente depois, ressuspendidos em 1 mL tampão de ureia (Ureia 6 M, tioureia 2 M, Tris-HCl 0,1M, pH 8 filtrada em filtros de membrada de celulose com porosidade de 0,22 µm), obtendo-se a Solução 1. Foi retirada uma alíquota de 50 µL da Solução 1 e diluiu-se em 950 µL de tampão ureia (Solução 2). A partir da Solução 2 foi realizado o ensaio de Bradford (Bradford, 1976) para determinação da concentração de proteínas. Após obter o teor de proteínas, foi realizada uma diluição a partir da Solução 1 para obter a concentração final de proteínas de 3,33 mg/mL, obtendo-se a Solução 3.

3.3.1.5.2 Digestão das proteínas

Uma alíquota de 15 µL foi retirada da Solução 3 (50 µm de proteínas/15 mL) e foi acrescentado 78 µL de bicarbonato de amônio 50 mM e 1 µL de ditiotreitol (DTT) as amostras, seguido de incubação a 56 °C por 20 min (redução). As amostras foram resfriadas a temperatura ambiente e, em seguida, foi adicionado 2,7 µL de iodoacetamida (IAA) 0,55 M e incubação por 15 min a temperatura ambiente, na ausência de luz (alquilação). Depois das etapas de redução e alquilação, foi acrescentado 1 µL de proteaseMAX e 1,8 µL de tripsina para cada uma das amostras, seguido de incubação a 37 °C por 4 horas. As amostras foram centrifugadas a 10.000 rpm por 50 s, em seguida, adicionado 1 µL de ácido fórmico, agitado em vórtex brevemente e incubado por 5 min a temperatura ambiente. As amostras foram centrifugadas a 13.000 rcf durante 10 min. Foi retirada uma alíquota de 80 µL do sobrenadante para um novo tubo, e desse, transferiu-se 30 µL a dois tubos eppendorf de 1,5 mL. Um dos tubos foi congelado (reserva de segurança), e o outro seguiu análise.

3.3.1.5.3 Limpeza das amostras digeridas

As amostras digeridas foram limpas usando ponteiras com colunas espirais C18/ZipTips fixadas na pipeta. A amostra digerida foi ressuspendida com 20 µL tampão ácido trifluoracético (TFA) 0,5% em água Milli-Q. Sonicou-se por 2 min para re-solubilização do precipitado, seguido de centrifugação (10.000 rpm por 50 s). A ponteira C18 foi umedecida com 10 µL de acetonitrila a 50% por duas vezes, sempre descartando a solução, em seguida, foi aspirado 10 µL do tampão TFA a 0,1% em água Milli-Q por duas vezes, e aspirou-se e dispensou-se lentamente 10 µL da amostra ressuspendida por 10 vezes (a ponteira permaneceu sempre imersa na solução contendo a amostra). Posteriormente, foi aspirado 10 µL de tampão TFA 0,1% em água MilliQ por duas vezes, descartando os resíduos, e foi aspirado e dispensado 10 µL de tampão TFA a 0,1% em acetonitrila a 60% em um novo eppendorf de 1,5 mL, este procedimento foi repetido uma vez mais. As amostras limpas foram secas em liofilizador SpeeddyVac (1.000 rpm a 30 °C) e armazenadas a -20 °C até análise. No dia da análise, as amostras limpas e secas foram ressuspendidas em 20 µL de tampão TFA a 0,05% em acetonitrila a 2%. Sonicou-se a suspensão por dois minutos e centrifugou-se por 5 min a temperatura ambiente. Foi recolhido 15 µL do sobrenadante e colocou-se no vial cônico, com o cuidado de não formar bolhas de ar.

3.3.1.5.4 Espectrometria de massa e identificação de proteínas

Um espectrômetro de massa Q-Exactive Plus acoplado a um Dionex Ultimate 3000 RSLCnano (Thermo Scientific) analisou 0,75 µg de cada digestão após a limpeza. Os gradientes de LC variaram de 5 a 45% de A (acetonitrila a 3%, ácido fórmico a 0,1%), B (acetonitrila a 80%, ácido fórmico a 0,1%) durante 2 h em uma coluna EASY-Spray, ID de 50 cm × 75 µm, PepMap RSLC C18, 2 µm (Thermo Scientific, CA, EUA). Os dados foram coletados usando um método Top15 para exames de MS/MS (Delgado et al., 2019, 2017; Dolan et al., 2014). A abundância comparativa de proteoma e a análise dos dados foram organizados e tratados estatisticamente nos softwares MaxQuant (Versão 1.6.0.13; www.maxquant.org/downloads.htm) (Cox & Mann, 2008) e Perseus (Versão 1.6.0.7). A carbamidometilação das cisteínas foi estabelecida como uma modificação fixa; a oxidação de metioninas e a acetilação dos terminais N foram definidas como modificações variáveis. A pesquisa no banco de dados foi realizada no banco de dados da proteína Gallus gallus (baixado em dezembro de 2018, www.uniprot.org). As taxas máximas de descoberta falsa de peptídeo/proteína (FDR) foram definidas em 1% com base na comparação com um banco de dados reverso. O algoritmo LFQ foi utilizado para gerar intensidades espectrais normalizadas e inferir abundância relativa de proteínas (Luber et al., 2010). As proteínas foram identificadas com pelo menos dois peptídeos, as proteínas que correspondiam a um banco de dados de contaminantes ou banco de dados reverso foram removidas e as proteínas foram retidas apenas na análise final se detectadas em pelo menos duas repetições de pelo menos um tratamento. A análise quantitativa foi realizada usando um teste t para comparar tratamentos com o controle. Apenas proteínas com uma alteração de dobra ≥ 2 ($p <0,05$) foram incluídas nos resultados quantitativos (Delgado et al., 2019, 2017; Dolan et al., 2014). A análise qualitativa também foi realizada para detectar proteínas encontradas em pelo menos três repetições do grupo WS-severo, mas indetectáveis no grupo de comparação.

3.3.2 Avaliação sensorial dos peitos N e WS

3.3.2.1 Teste de aceitabilidade e intenção de compra

Um total de 101 consumidores e compradores regulares de carne de frango participaram do estudo. Os julgadores foram recrutados entre funcionários e estudantes da Universidade da Extremadura (Cáceres, Espanha). Os testes foram realizados em duas etapas, simulando condições de compra/venda de peitos de frango nas prateleiras dos supermercados. As amostras foram apresentadas na forma de meio filé de peito cru, individualmente dispostas em bandejas envolvidas com plástico filme, sob condição de temperatura refrigerada (as bandejas foram mantidas sobre gelo triturado durante toda avaliação). As amostras foram colocadas em duas mesas, uma para cada etapa, de maneira que não houvesse troca de informações, para não comprometer o julgamento dos avaliadores. Cada mesa continha 2 peitos normais, 2 WS-moderado e 2 WS-severo.

Na primeira etapa, os consumidores realizaram uma avaliação “cega” das amostras, ou seja, nenhuma informação sobre a miopatia *White Striping* foi fornecida. Nesta etapa as amostras foram apresentadas codificadas com números de 3 dígitos, sem qualquer informação adicional. Na segunda etapa, os consumidores realizaram uma avaliação “informada” das amostras. Aos participantes, foram apresentados peitos Normal, WS-moderado, e WS-severo, também codificados com números de 3 dígitos, porém, foi fornecida informação sobre a miopatias e seus diferentes graus de estrias (APÊNDICE A).

A aceitabilidade e a intenção de compra foram avaliadas pelos consumidores com base na aparência visual da carne crua de peito de frango usando uma escala hedônica não-estruturada de cinco pontos, variando de 1 'gostei extremamente' ou 'definitivamente não compraria', a 5 ' gostei extremamente 'ou' definitivamente compraria ' (APÊNDICE B).

3.3.2.2 Perfil emocional do consumidor

O estudo foi realizado com 46 consumidores regulares de carne de frango recrutados na Universidade de Extremadura (Cáceres, Espanha). Os provadores mediram as respostas emocionais referentes ao consumo de carne de frango usando o questionário *rate-all-that-apply* (RATA) e teste de aceitabilidade com escala facial. Foram selecionados para o experimento vinte peitos de frango. A seleção foi baseada na presença ou ausência de estriação branca na superfície do músculo (10 Normal e 10 WS-severo). Os peitos de frango congelados (-18 ° C por 48 h) foram envolvidos em papel alumínio e aquecidos no forno a 180 ° C até atingir uma temperatura interna de 75 ° C. Posteriormente, as carnes foram cortadas em cubos de 1,5 cm e submetidas à análise sensorial sob duas condições experimentais.

No experimento 1, foi solicitado aos consumidores que avaliassem suas respostas emocionais e aceitabilidade sob condição não informada, sendo as amostras apresentadas codificadas com números aleatórios de 3 dígitos. No experimento 2, as mesmas análises foram realizadas, no entanto, os consumidores avaliaram as amostras após um breve relato sobre a miopatia WS (APÊNDICE C). Neste experimento, as amostras foram apresentadas identificadas e sem código. Em ambos os experimentos, foi solicitado aos consumidores que verificassem os termos que consideravam apropriados para descrever amostras e, em seguida, classificassem a intensidade dos termos aplicáveis usando uma escala de cinco pontos. A lista de termos foi desenvolvida com base nos atributos emocionais selecionados por avaliadores treinados, a partir de uma lista inicial de 39 termos descritos por Dorado et al. (2016).

Pediu-se aos provadores que avaliassem a aceitabilidade das amostras em sua aparência, odor, sabor, textura e impressão global usando uma escala facial de 5 pontos, de 1 = desgostei extremamente a 5 = gostei extremamente (APÊNDICE D), e respondessem ao questionário RATA composta de 25 termos emocionais (ativo, aventureiro, agressivo, entediado, enojado, entusiasmado, livre, alegre, bem, bem-humorado, culpado, feliz, interessado, amoroso, nostálgico, quieto, agradável, satisfeito, seguro, tranquilo, terno, compreensivo, zangado, furioso, preocupado), usando uma escala de intensidade de 5 pontos ancorada de "nada" a "extremamente" (APÊNDICE E).

3.4 AVALIAÇÃO ESTATÍSTICA

Os dados obtidos nos estudos envolvendo a caracterização físico-química e sensorial dos peitos, foram submetidos ao teste de normalidade Shapiro-Wilk ($\alpha = 0,05$). As amostras tidas como normais (distribuição gaussiana) foram submetidas a Análise de Variância (ANOVA) e as médias comparadas por Tukey ($p < 0,05$). Para as amostras não normais foi aplicado o teste não paramétrico de Kruskal-Wallis ($p < 0,05$) e as médias comparadas entre si pelos testes de Dunn's com o nível alfa de 0,05 para comparar três ou mais amostras, e de U de Mann-Whitney ($p<0,05$) para comparar duas amostras. As respostas obtidas no questionário RATA (Rate-All-That-Apply) foram avaliados pelo teste Cochran's Q ($p<0,05$). A análise estatística e gráficos utilizados foram gerados pelos programas GraphPad Prism (versão 6.0 para Windows, Graphpad Software Inc., San Diego, California, USA) e XLSTAT (versão 2014.5.03, Addinsoft, New York, USA).

REFERÊNCIAS

ABPA (Associação Brasileira de Proteína Animal). **Relatório Anual 2020**. São Paulo: ABPA, 2020, 158 p. Disponível em: <http://abpa-br.org/relatorios/>. Acesso em: 30 maio 2020.

AEBI, H. E. Catalase. In: H. U. BERGMEYER (Ed.). **Methods for enzymatic analysis** (Vol 1). Weinheim: Verlag Chemie, pp. 273-286, 1974.

AOAC (2000). **Official Methods of Analysis**. Gaithersburg, Maryland, USA: Association of Official Analytical Chemists. Methods 981.10, 950.46, 990.26.

BAILEY, R. A.; WATSON, K. A.; BILGILI, S. F.; AVENDANO, S. The genetic basis of pectoralis major myopathies in modern broiler chicken lines. **Poultry Science**, v.0, p.1-10, 2015.

BOULIANNE, M.; KING, J. Meat color and biochemical characteristics of unacceptable Dark-colored broiler chicken carcasses. **Journal of Food Science**, v.63, n.5, p.759-762, 1998.

BRADFORD, M.M. A rapid and sensitive method for the determination of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry,** v.72, p. 248-254, 1976.

CARVALHO, R. H.; IDA, E. I., MADRUGA, M. S.; MARTÍNEZ, S. L.; SHIMOKOMAKI, M.; ESTÉVEZ, M. Underlying connections between the redox system imbalance, protein oxidation and impaired quality traits in pale, soft and exudative (PSE) poultry meat. **Food Chemistry**, v.215, p.129-137, 2017.

CHATTERJEE, D.; ZHUANG, H.; BOWKER, B.C.; RINCON, A.M.; SANCHEZ-BRAMBILA, G. Instrumental texture characteristics of broiler pectoralis major with the wooden breast condition. **Poultry Science**, v.95, n.10, p.2449-2454, 2016.

CHELH, I.; GATELLIER, P.; SANTÉ-LHOUTELLIER. Characterisation of fluorescent Schiff bases formed during oxidation of pig myofibrils. **Meat Science**, v.76, p.210-215, 2007.

CIE. **CIE Publication 15.2**. Vienna: Commission Internationale de l'Eclairage; 1986.

COX, J.; MANN, M., 2008. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. **Nature Biotechnology**, v.26, p.1367-1372, 2008.

DELGADO, J.; OWENS, R. A.; DOYLE, S.; NÚÑEZ, F.; ASENSIO, M. A. Quantitative proteomics reveals new insights into calcium-mediated resistance mechanisms in Aspergillus flavus against the antifungal protein PgAFP in cheese. **Food Microbiology**, v.66, p.1-10, 2017.

DELGADO, J.; NÚÑEZ, F.; ASENSIO, M. A.; OWENS, R. A. Quantitative proteomic profiling of ochratoxin A repression in Penicillium nordicum by protective cultures. **International Journal of Food Microbiology**, v.305, 108243, 2019.

DELLES, R. M.; XIONG, Y. L.; TRUE, A. D.; AO, T.; DAWSON, K.A. Dietary antioxidant supplementation enhances lipid and protein oxidative stability of chicken broiler meat through promotion of antioxidant enzyme activity. **Poultry Science**, v.93, p.1561-1570, 2014.

DOMÍNGUEZ, R.; PATEIRO, M.; GAGAOUA, M.; BARBA, F.J.; ZHANG, W.; LORENZO, J. M. A comprehensive review on lipid oxidation in meat and meat products. **Antioxidants**, n.8, v.10, p.429.

DOLAN, S. K.; OWENS, R. A.; O'KEEFFE, G.; HAMMEL, S.; FITZPATRICK, D. A.; JONES, G. W.; DOYLE, S. Regulation of Nonribosomal Peptide Synthesis: bis-Thiomethylation Attenuates Gliotoxin Biosynthesis in Aspergillus fumigatus. **Chemistry & Biology**, v.21, n.8, p.999-1012, 2014.

DORADO, R.; PÉREZ-HUGALDE, C.; PICARD, A.; CHAYA, C. Influence of first position effect on emotional response. **Food Quality and Preference**, v.49, p.189-196, 2016.

ESTÉVEZ, M.; LUNA, C. Dietary protein oxidation: A silent threat to human health? C**ritical Reviews in Food Science and Nutrition**, v.57, n.17, p.3781-3793, 2016.

FAOSTAT. **Download Data: Livestock Primary**. 2019. Disponível em: <http://www.fao.org/faostat/en/#data/QL>. Acesso em: 3 abr. 2020.

FOLCH, J.; LEES, M.; STANLEY, G. H. S. A Simple method for the isolation and purification of total lipids from animal tissues. **Journal of Biological Chemistry**, v.226, n.1, p.497-509, 1957.

GANHÃO, R.; ESTÉVEZ, M.; MORCUENDE, D. Suitability of the TBA method for assessing lipid oxidation in a meat system with added phenolic-rich materials. **Food Chemistry**, v.126, n.2, p.772-778, 2011.

HALLIWELL, B.; AESCHBACH, R.; LOLIGER, J.; ARUOMA, O. I. The Characterization of antioxidants. **Food Chemistry Toxicology**, v.33, n.7, p.601-617, 1995.

KUTTAPPAN, V. A.; LEE, Y. S.; ERF, G. F.; MEULLENET, J. F. C.; MCKEE, S. R.; OWENS, C. M. Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. **Poultry Science**, v.91, p.1240-1247, 2012a.

KUTTAPPAN, V. A.; BREWER, V. B.; APPLE, J. K.; WALDROUP, P. W.; OWENS, C. M. Influence of growth rate on the occurrence of white striping in broiler breast fillets. **Poultry Science**, v.91, p.2677-2685, 2012b.

LUBER, C.A.; COX, J.; LAUTERBACH, H.; FANCKE, B.; SELBACH, M.; TSCHOPP, J.; AKIRA, S.; WIEGAND, M.; HOCHREIN, H.; O'KEEFFE, M.; MANN, M.

Quantitative Proteomics Reveals Subset-Specific Viral Recognition in Dendritic Cells. **Immunity**, v.32, n.2, p.279-289, 2010.
LUND, M. N.; HEININEN, M.; BARON, C. P.; ESTÉVEZ, M. Protein oxidation in muscle foods: A review. **Molecular Nutrition & Food Research**, v.55, n.1, p.83-95, 2011.

MALILA, Y.; U-CHUPAJ, J.; SRIMARUT, Y.; CHAIWIWATTRAKUL, P.; UENGWETWANIT, T.; ARAYAMETHAKORN, S.; PUNYAPORNWITHAYA, V.; SANSAMUR, C.; KIRSCHKE, C. P.; HUANG, L.; TEPAAMORNDECH, S.; PETRACCI, M.; RUNGRASSAMEE, W.; VISESSANGUAN, W. Monitoring of white striping and wooden breast cases and impacts on quality of breast meat collected from commercial broilers (*Gallus gallus*). **Asian-Australas Journal of Animal Science**, v.31, n.11, p.1807-1817, 2018.

MARKLUND, S.; MARKLUND, G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. **European Journal of Biochemistry**, v.47, p.469-474, 1974.

MORRISSEY, P. A.; SHEEHY, P. J.; GALVIN, K.; KERRY, J. P.; BRUCKLEY, D. J. Lipid stability in meat and meat products. **Meat Science**, v.49, n.1, p.73-86, 1998.

MUDALAL, S.; LORENZI, M.; SOGLIA, F.; CAVANI, C.; PETRACCI, M. Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. **Animal**, v.9, n.4, p. 728-734, 2015.

OOIZUME, T.; XIONG, Y. Hydroxyl radical oxidation destabilizes subfragment-1 but not the rod of myosin in chicken myofibrils. **Food Chemistry**, v.106, n.2, p.661-668, 2008.

PETRACCI, M.; CAVANI, C. Muscle Growth and Poultry Meat Quality Issues. **Nutrients**, v.4, p.1-12, 2012.

PETRACCI, M.; MUDALAL, S.; BONFIGLIO, A.; CAVANI, C. Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. **Poultry Science**, v.92, p.1670-1675, 2013.

PETRACCI, M.; SOGLIA, F.; MADRUGA, M.; CARVALHO, L.; IDA, E.; ESTÉVEZ, M. Wooden-Breast, White Striping, and Spaghetti Meat: Causes, consequences and consumer perception of emerging broiler meat abnormalities. **Comprehensive Reviews in Food Science and Food Safety**, v.18, n.2, p.565-583, 2019.

POPOVA, T.; MARINOVA, P.; VASILEVA, V.; GORINOV, Y.; LIDJI, K. Oxidative changes in lipids and proteins in beef during storage. **Archiva Zootechnica**, v.12, n.3, p.30-38, 2009.

RYSMAN, T. JONGBERG, S.; ROYEN, G. V.; WEYENBERG, S.V.; SMET, S. D.; LUND, M.N. Protein Thiols Undergo Reversible and Irreversible Oxidation during Chill Storage of Ground Beef as Detected by 4,4′-Dithiodipyridine. **Journal of Agricultural and Food Chemistry**, v.62, p.12008-12014, 2014.

SIHVO, H. K.; IMMONEN, K.; POULANNE, E. Myodegeneration with fibrosis and regeneration in the Pectoralis major muscle of broilers. **Veterinary Pathology, v.**51, n.3, p.619-23, 2014.

SILVA, C.S.; BORBA, F.S.L.; PIMENTEL, M.F.; PONTES, M.J.C.; HONORATO, R.S.; PASQUINI, C. Classification of blue pen ink using infrared spectroscopy and linear discriminant analysis. **Microchemical Journal**, v.109, p.122-127, 2013.

SOGLIA, F.; LAGHI, L.; CANONICO, L.; CAVANI, C.; PETRACCI, M. Functional property issues in broiler breast meat related to emerging muscle abnormalities. **Food Research International,** v.89, n.3, p.1071-1076, 2016.

TICKLE, P. G.; PAXTON, H.; RANKIN, J. W.; HUTCHINSON, J. R.; CODD, J. R. Anatomical and biomechanical traits of broiler chickens across ontogeny. Part I. Anatomy of the musculoskeletal respiratory apparatus and changes in organ size. **PeerJ**, v.2(e432), p.1-17, 2014.

UTRERA, M.; MORCUENDE, D.; RODRÍGUEZ-CARPENA, J. G.; ESTÉVEZ, M. Fluorescent HPLC for the detection of specific protein oxidation carbonyls – α-aminoadipic and γ-glutamic semialdehydes – in meat systems. **Meat Science**, v.89, p.500-506, 2011.

VILJANEN, K.; KIVIKARI, R.; HEINONEN, M. Protein-lipid interactions during liposome oxidation with added anthocyanin and other phenolic compounds. **Journal Agricultural of Food Chemistry**, v.52, p.1104-1111, 2004.

XIAO, S., W. G. ZHANG, E. J. LEE, C. W. MA, AND D. U. AHN. Effects of diet, packaging, and irradiation on protein oxidation, lipid oxidation, and color of raw broiler thigh meat during refrigerated storage. **Poultry Science**, v.90, p.1348-1357, 2011.

ZHU, X.; RUUSUNEN, M.; GUSELLA, M.; ZHOU, G.; PUOLANNE, E. High post-mortem temperature combined with rapid glycolysis induces phosphorylase denaturation and produces pale and exudative characteristics in broiler pectoralis major muscles. **Meat Science**, v.89, p.181-188, 2011.

ZUIDHOF, M. J.; SCHNEIDER, B. L.; CARNEY, V. L.; KORVER, D. R.; ROBINSON, F. E. Growth, efficiency, and yield of commercial broilers from 1957, 1978, and 2005. **Poultry Science**, v.93, p.1-13, 2014.

4 RESULTADOS E DISCUSSÃO

Os resultados obtidos nesta pesquisa estão apresentados no formato de artigo, em atendimento a Norma Complementar nº 03/2011 do PPGCTA.

4.1 ARTIGO I – NEAR-INFRARED SPECTROSCOPY AND MULTIVARIATE ANALYSIS TO IDENTIFY CHICKEN BREASTS AFFECTED BY WOODEN BREAST AND WHITE STRIPPING MYOPATHIES IN BRAZILIAN SLAUGHTERING PLANTS

O artigo foi submetido ao periódico Journal of Food Science - IFT em 06 de maio de 2020, sob o título *Near-infrared Spectroscopy and Multivariate Analysis to Identify Chicken Breasts Affected by Wooden Breast and White Stripping Myopathies in Brazilian Slaughtering Plants* (ANEXO B).

Near-infrared Spectroscopy and Multivariate Analysis to Identify Chicken Breasts Affected by Wooden Breast and White Stripping Myopathies in Brazilian Slaughtering Plants

Leila Moreira de Carvalho[1], Marta Suely Madruga[1], Mario Estévez[2], Amanda Teixeira Badaró[3], Douglas Fernandes Barbin[3]

[1] Department of Food Engineering, Federal University of Paraiba, João Pessoa, PB, Brazil.

[2] Institute of Meat and Meat Products (IPROCAR), TECAL Research Group, University of Extremadura, Cáceres, Spain.

[3] Department of Food Engineering, University of Campinas, Campinas, SP, Brazil.

Abstract

White Striping (WS) and Wooden Breast (WB) are emerging poultry myopathies that occur worldwide, affecting the quality of meat. WS is characterized by the presence of stretch marks on the muscle surface, while WB is characterized by the substantial hardness of the breast muscle. The aim of this study was to assess the suitability of applying Near-infrared Spectroscopy (NIRS) to identify WS and WB myopathies by comparing the results from those obtained using traditional inspection based on visual aspect and palpation of *Pectoralis major* muscle. Chickens slaughtered at Brazilian commercial plant at four age ranges (4-5, 6-7, 8-9 and 65 weeks) were inspected. Spectral information was acquired using a portable NIR spectrometer, and classification models were performed using and Successive

Projection Algorithm-Linear Discriminant Analysis (SPA-LDA) and Soft Independent Modeling of Class Analogy (SIMCA) to distinguish normal and affected muscles. Results showed that occurrence of myopathies was aggravated by age of slaughter, as chicken slaughtered at 4-5 and 65 weeks exhibited 13.6% and 95 % of myopathies, respectively. Birds slaughtered at 65 weeks showed no occurrence of WB, isolated or combined with WS. SPA-LDA model showed greater accuracy (92-93%) in identifying N, WS and WB+WS/WB groups, compared to SIMCA (89-91%). It can be concluded that the level of occurrence of myopathies in meat is directly related to the age of slaughter. This study demonstrated that NIRS combined with SPA-LDA model can be used as a tool to detect myopathies in chicken breast. This technique has potential for application in industrial processing lines as an alternative to the traditional methods of identification.

Keywords: Poultry meat, Myopathies, Linear discriminant analysis, Successive projection algorithm, Soft independent modeling of class analogy.

Introduction

Chicken meat production worldwide increased from around 54.4 million tonnes in 1998, to 114.2 million tonnes in 2018. In that same period, Brazil showed a 307% increase in chicken production, from 4.9 to 14.9 million tonnes (FAOSTAT, 2019). As a result, Brazil became the biggest exporter and second largest producer of poultry in the world (Brazilian Association of Animal Protein, 2019).

This increase in poultry meat production was made possible by the intense genetic selection of birds, which provided an increase in feed efficiency and growth rate of the birds (Barbut et al., 2008; Paxton, Anthony, Corr, & Hutchinson, 2010; Tickle, Paxton, Rankin, Hutchinson, & Codd, 2014). However, an accelerated growth rate had led to altered physiological processes (Schmidt, Persia, Feierstein, Kingham, & Saylor, 2009; Tickle et al., 2014), resulting in the occurrence of myopathies (MacRae, Gilpin, Sandercock, Hunter, & Mitchell, 2007; Petracci, & Cavani, 2012). Some of these emerging myopathies include White Striping (WS) and Wooden Breast (WB). WS is characterized by the presence of white streaks on the surface of the muscle tissue that follow the direction of the fibers (Petracci et al., 2012). WB is marked by the hardness of the breast muscle and may present hemorrhage foci and exudate on its surface (Bailey, Watson, Bilgili & Avendano, 2015). These two conditions can also be observed in a combined way (WS/WB).

Birds affected by WS, WB and WS/WB have been reported in several countries, with values up to 95% occurrence. The occurrence of WS ranged from 44-72% of animals in Turkey (Adabi & Soncu, 2019), 48-63% in USA (Kuttappan et al., 2009), 89-95% in Thailand (Malila et al., 2018) and 10-82% in Italy (Petracci, Mudalal, Bonfiglio, & Cavani, 2013; Russo, Drigo, Longoni, Pezzotti, Fasoli, & Recordati, 2015). The occurrence of WB meat varied from 8-16% in Italy (Trocino et al., 2015) and WS/WB from 7-8% in Thailand (Malila et al., 2018).

In general, the identification of these myopathies is performed post-mortem through visual examination and/or palpation of the breast muscle (Mutryn, Brannick, Fu, Lee, & Abasht, 2015; Sihvo, Lindén, Airas, Immonen, & Poulanne, 2016). This method requires a considerable number of trained evaluators, and the sensitivity of detection varies from one individual to another. For this reason, non-destructive and rapid instrumental methods are being studied as an alternative to the traditional method of identification, since they are more convenient, accurate and effective than analysis based on appearance and palpation (Petracci, Soglia, Madruga, Carvalho, Ida, & Estévez, 2019).

Near infrared spectroscopy has been widely applied for rapid, sensitive and non-destructive food analyses (Alander, Bochko, Martinkauppi, Sarawong & Mantere, 2013). In addition, NIR spectroscopy can be used in industrial processing lines for the classification/authentication of samples (Barbin, ElMasry, Sun, & Allen, 2013; Perez, Badaró, Barbon, Barbon, Pollonio, & Barbin, 2018). Multivariate statistical analyses are necessary to extract useful information from NIR spectra, and thus have been applied to distinguish the different samples or concentration of specific chemical compounds in chicken (Geronimo et al., 2019; Wold, Veiseth-Kent, Host, & Lovland, 2017).

Brazil is the second largest broiler producer and chicken-meat exporter worldwide. However, in contrast to other countries, there is little information available on the occurrence of WS and WB myopathies in Brazil.

The present study proposed to detect and quantify the occurrence of WS and WB in Brazilian commercial plants using traditional detection by visual method, and evaluate the potential use of a portable near-infrared spectrometer together with multivariate statistical analysis to distinguish normal muscles from muscles affected by WB and WS myopathies.

Material and methods

Experimental design

Two experiments were conducted in a Brazilian slaughterhouse on birds from the Cobb lineage of different slaughter age ranges. Samples were collected from different producers over a four-month period, for both experiments, to have a representative and unbiased data set.

In experiment 1, a total of 8,959 birds, classified according to slaughter age (weeks) as 4-5 (G1; n=1,200), 5-6 (G2; n=6,919), 7-8 (G3; n=600) and 65 weeks (G4; n=240), were evaluated based on the visual aspect and palpation of *Pectoralis major* muscle for occurrence of WS and WB myopathies.

The number of birds analyzed represented 10% of animals daily slaughtered in the commercial plant, taking into account the age range of the bird slaughtered. The muscles were classified by the traditional method according to the severity of myopathies (Figure 1). WS myopathy was classified into 2 categories: moderate (thickness < 1 mm) and severe (thickness > 1 mm). WB myopathy was classified into 3 categories: mild (focal hardness in cranial region), moderate (moderate and extensive hardness) and severe (extreme and diffuse hardness). WS/WB was classified into 6 categories based on the association between WS and WB categories.

Samples were also classified by the traditional method according to the extension of myopathies: WS into 2 categories according to the surface area of the muscle affected by the stretch marks (< 1/2, or > 1/2 of affected muscle surface area); WB and WS/WB were classified into 2 categories, according to presence of hemorrhage in the muscle surface (with or without hemorrhage). In addition, a total of thirty-two breast samples were collected from 6-7 weeks slaughtered broilers to determine the proximate composition (N, n = 8; WS, n = 8; WB, n = 8; and WS/WB, n = 8).

In Experiment 2, a total of 1,028 of *P. major* muscle from slaughtered birds with different age ranges unaffected by any myopathies (normal (N), n=271; 4-5 week: 40; 6-7 week: 204; 8-9 week: 12; 65 week: 12) and affected by WS (n=272; 4-5 week: 40; 6-7 week: 205; 8-9 week: 15; 65 week: 12), WB (n=249; 4-5 week: 30; 6-7 week: 204; 8-9 week: 15), WS/WB (n=236; 4-5 week: 17; 6-7 week: 204; 8-9 week: 15) myopathies were analyzed in the processing line using a portable near-infrared spectroscopy. The number of birds analyzed by NIR spectroscopy was defined as approximately 1% of daily slaughtering carcasses of the commercial plant, considering the age range of the bird slaughtered.

Proximate composition

Moisture was determined by oven-drying at 105 °C, as described by AOAC (2000). Total nitrogen was determined by Kjeldahl method, considering the conversion factor of 6.25 for crude protein, according to AOAC (2000). Total lipids were determined by the method of Folch, Less, and Stanley (1957) and collagen content was determined according to the methodology of AOAC (2000).

NIR spectra measurements

A portable spectrophotometer (DLP NIRscan Nano EVM, Texas Instruments, Dallas, Texas) was employed to obtain NIR spectra in the range of 900 to 1,700 nm. All spectra were recorded with an average of 10 scans per sample, in absorbance mode. Room temperature was controlled at 12±2 °C throughout the spectral acquisition process. The spectra acquisition was performed in the external surface of the cranial region of *Pectoralis major* muscle.

Spectral data processing

The NIR spectra were used to build classification models using SPA-LDA (Successive Projection Algorithm-Linear Discriminant Analysis) and SIMCA (Soft Independent Modeling of Class Analogy). The raw data were pre-processed through Savitzky-Golay smoothing, 1st derivative and 2nd derivative, Standard Normal Variate (SNV) and Multiplicative Scatter Correction (MSC). Principal component analysis (PCA) was used for sample discrimination. Score, residual and leverage plots were employed in the detection and elimination of outliers using Robust PCA (RPCA). The pre-processed data were separated into: training (60% of samples), validation (20% of samples) and test (20% of samples) sets using the Kennard-Stone algorithm (Silva, Borba, Pimentel, Pontes, Honorato, & Pasquini, 2013). The Kennard-Stone (KS) algorithm was applied separately to each class. Chemometric data treatment was implemented with Matlab (R2010a, version 7.10.0.499, The Mathworks, Inc., Natick, MA) software equipped with a multivariate statistics package.

Statistical analyses

The physical-chemical data were submitted to analysis of variance, and the means of the treatments were compared by Tukey test ($p < 0.05$). The data analysis was performed using XLStat software (XLStat version 138 2014.5.03, Addinsoft, New York, USA).

Results and Discussion

Occurrence of WS and WB myopathies

Figure 2 shows the occurrence of myopathies in *Pectoralis major* muscle of birds with different slaughter ages range. The higher occurrence of myopathies was observed in breasts from birds slaughtered at 65 weeks-old (95%) and 8-9 weeks-old (84.7%), while breast muscles from birds slaughtered at 6-7 and 4-5 weeks-old exhibited 57.0%, and 13.6% of myopathies, respectively. These results demonstrate a greater occurrence of myopathies in meat of older birds, confirming the findings of Adabi et al. (2019) who observed an increase from 44% to 71% of myopathies in birds aged 3-4 to 5-6 weeks, respectively.

Furthermore, the age also leads to a considerable increase in the occurrence of WS myopathy, i.e. from 9.6% to 95% in 4-5 and 65 weeks-old birds, respectively. In their study, Dalle Zotte, Tasoniero, Russo, Longoni, and Cecchinato (2015) did not detect the presence of stretch marks in chicken breasts aged 1-2 weeks. However, they observed a prevalence of 5.4% of WS in broiler breast meat aged 3-4 weeks and 98.4% in broiler aged 7-8 weeks.

WB occurrence was detected at 2.6, 11.2 and 5.7% in birds aged 4-5, 6-7, 8-9 weeks, respectively. This reduction in the age of 8-9 weeks, may be related to the increase of live weight of birds with increasing age at slaughter, making the breasts from these animals more prone to the occurrence of WB meat associated with WS myopathy, rather than isolated.

WS/WB breasts was also aggravated by the increase in slaughtering age, as the occurrence increased from 1.4% in birds aged 4-5 weeks to 27% in birds aged 8-9 weeks. WB and WS/WB myopathies were not observed in birds slaughtered at 65 weeks-old (mother hens). As these birds are geared towards the broilers production, they are not subjected to rapid muscle growth in a short period of time, so they are less likely to develop WB myopathy. According to Velleman, and Clark (2015), WB condition affects the muscle of commercial broiler lines with fast muscle growth.

Figure 3 shows the categories description (degree and extension) for breast meat with WS, WB and WS/WB myopathies. WS breasts showed a predominance of moderate degree stretch marks at all slaughter ages (Fig. 3A). In addition, the occurrence of moderate (9.6 vs. 28.1 vs. 50.2 vs. 92.5%) and severe (0.0 vs. 0, 7 vs. 1.8 vs. 2.5%) WS increased along with the age of the birds. Severe WS not was detected among birds slaughtered at 4-5 weeks. The birds slaughtered at 4-5 and 6-7 weeks showed a greater predominance of stretch marks that covered less than half of the muscle surface, 7.6 and 23.2%, respectively (Fig. 3B). Birds slaughtered at 8-9 and 65 weeks of age showed greater area of the muscular surface covered by stretch marks (44.0 and 76.3%, respectively). In general, stretch marks extended from the cranial to caudal region, occupying the entire muscle surface.

Alnahhas et al. (2016) observed an occurrence of 50.7% of breasts with stretch marks, among which 36.7% had moderate and 14% severe degree. Kuttappan, Brewer, Apple, Waldroup, and Owens (2012) found that in high performance poultry with a slaughter age of 54 days, the moderate and severe WS was 65.9% and 8.7%, respectively. The worsening of myopathies with increasing slaughtering age has also been observed in other studies. Dalle Zotte et al. (2015) observed a prevalence of 5.4% of moderate and 0% severe degree in broiler slaughtered at 25 days, whereas poultry with commercial slaughter age (51 days) presented 36.8% of moderate and 61.6% severe stretch marks. Lorenzi, Mudalal, Cavani, and Petracci (2014) pointed out that the occurrence of WS in poultry aged 51-50 days was higher for the moderate (46.9% versus 25.8%) and severe (9.5% versus 2.7%) degree compared to 41-40-day old poultry.

Birds slaughtered at 4-5 weeks showed a higher proportion of breasts with a moderate WB (2.2%) (Fig. 3C). Birds slaughtered at 6-7 weeks presented a higher occurrence of breasts with mild degree (localized hardness in the cranial region), followed by moderate and severe degrees (4.4 vs. 4.1 vs. 2.7%, respectively). Birds slaughtered at 8-9 weeks had a larger proportion of breasts with a severe degree of hardness and followed by breasts with moderate and mild degrees (3.7 vs. 1.6 vs. 0.2%, respectively). The occurrence of severe WB increased progressively with the age of slaughter (0.3 vs. 2.7, vs. 3.7%). Furthermore, the percentage of WB breasts with hemorrhage in the muscle surface was 0.1%, 2.2% and 1.2% in birds slaughter at 4-5, 6-7 and 8-9 weeks old, respectively (Fig. 3D). Tijare, Yang, Kuttappan, Alvarado, Coon, and Owens (2016) observed an occurrence of 91% WB, of these 48% mild, 28% moderate and 20% severe. These values reported were extremely higher as compared to those observed in the present study.

The severity degree of WS/WB is shown in Figure 3E. The birds slaughtered at 4-5 weeks old exhibited a higher occurrence (0.5%) of breasts with moderate and extensive hardness in addition to stretches of thickness less than 1 mm (WS/WB-3: WB-M and WS-M). In addition, there was no occurrence of WS/WB-2 breasts (WB-I and WS-S). Birds aged 6-7 weeks presented a higher percentage (6.3%) of breasts with localized hardness in the cranial region and stretch marks of thickness <1 mm (WS / WB-1: WB-I and WS-M). Yet, birds aged 8-9 weeks showed a higher occurrence (10.2%) of the WS/WB-6 (WB-S and WS-S), that is, breasts with diffuse and extreme hardness and streak marks with thickness greater than 1 mm. The increase in age at slaughter led to increase of WS/WB breast with hemorrhages in surface, from 0.2 to 7 % (Fig. 3F). These results demonstrate that with increasing age of slaughter, the birds are more likely to develop the most severe degrees of WB and WS myopathies, in addition to being more likely to develop hemorrhages on the muscle surface. Malila et al. (2018) observed that broilers slaughtered at 6 weeks showed

5% WS/WB-mild and 2% WS/WB-moderate, while birds slaughtered at 7 weeks had 2% WS/WB-mild, 4% WS/WB-moderate and 2% WS/WB-severe, clearly demonstrating the worsening of myopathy with increasing poultry age.

Proximate composition

Table 1 shows the results of proximate composition of the poultry breasts. No significant differences in moisture content were observed among samples. WS, WB and WS/WB breast meats had the lowest protein content ($p = 0.0161$), and a higher content of lipid ($p = 0.0001$) and collagen ($p < 0.0001$), compared to Normal meat. However, no significant difference was found among the myopathies WS, WB and WS/W for these parameters. Variations in protein, lipid and collagen contents are associated to the muscle damage that occurs during the onset of these myopathies. Protein content decrease can be explained by the degeneration and atrophy of muscle fibers, while the increase of fat and collagen contents are related to the injured area replacement with fat (lipidosis) and connective tissue (fibrosis) (Kuttappan et al., 2013; Petracci, Mudalal, Babini, & Cavani, 2014; Mudalal, Babini, Cavani, & Petracci, 2014; Sihvo, Immonen, & Poulanne, 2014; Mutryn et al., 2015).

NIR spectra

Figure 4 shows the raw NIR spectra acquired from four classes (N, WS, WB, WS/WB) in the range 900-1700 nm. Spectra from normal and affected muscles exhibited similar shape but different absorption intensities. Normal breast presented higher absorbances (900 to 1390 nm) than WS, WB and WS/WB samples. Such results are reasonable due the difference in the chemical composition, specially protein, lipid and collagen content, observed between these samples.

Some bands were recorded at approximately 970 nm, 1150 nm and 1450 nm in the breast samples. In general, the absorptions observed in the near infrared region between wavelengths at 950-1100 nm are related with the second overtone of N-H and O-H stretching (Peng & Wang, 2015, Perez et al., 2018), while bands at 1140-1160 nm are influenced by absorption exerted by the second overtone of alkenes (C-H stretch) and fourth overtone C=O stretching (Eldin, 2011, Marques et al., 2015), and peaks at approximately 1440-1460 nm are associated with C-H combination, the first overtone N-H stretching, first overtone O-H stretching of water and third overtone C=O stretching (Eldin, 2011; Perez et al., 2018).

Principal component analysis

PCA was applied after data pre-processing (Savirtzky-Golay smoothing, first and second derivative, SNV and MSC). Figure 5 shows the PCA scores, with samples identified according to slaughter age. Based on visual inspection of the PCA scores plots, there was an overlap of classes (slaughter age) for all types of samples (N, WS, WB or WS/WB) and pre-processing conditions analyzed. Thus, regardless of the type of breast condition (Normal or affected muscles), the four age groups appeared to be evenly intermixed. This indicate a similar feature of spectrum for all slaughtering age groups, making it possible to treat all age range as a single class.

Figure 6 details the graphical representation of the PCA scores of NIR pre-processed spectra for four or three sample classes, according to the breast condition (normal or affected by WS and WB myopathy). As can be seen in Figure 6A1-A6, the WB and WS/WB classes are completely overlapping, with no distinct clustering between samples. However, there was a separation between theses samples and N and WS breast. This result indicates that the chemical changes caused by WB myopathy are dominant over WS. Thus, considering that the WB and WS/WB samples share the same myopathy (wooden breast), those samples were grouped (WB+WS/WB) for the purpose of improving sample separation.

Figure 6B1-B6 shows the samples clustering related to the three classes: N, WS, WB+WS/WB. It is possible to observe a trend of separation, with few samples overlapping. The cumulative variance for PCs the had most influence in separation was 98.8% for smoothing (first and second PCs), 82.3% for smoothing and 1st derivative (first and second PCs), 34.7% for smoothing and 2nd derivative (first and fourth PCs), 70.3% for smoothing and SNV (first and third PCs), 70.5% for smoothing and MSC (first and third PCs) pretreatments. The breasts presented satisfactory separation of classes, probably because of differences in chemical composition observed between these classes.

SPA-LDA and SIMCA classification

Both SPA-LDA and SIMCA models were used for building classification/discriminant models. Table 2 shows the overall performance results obtained by models applied to the calibration and prediction set using the pre-treated data. In both models (SPA-LDA and SIMCA), the best prediction results were achieved with the smoothing, and smoothing associated with the first derivative pre-processing data.

SPA-LDA results for the prediction set were similar when NIR spectra were pre-processed using smoothing and smoothing associated with the first derivative, with an accuracy of 92% and 93%, respectively (Table 2). On the other hand, when the smoothing associated with the second derivative of Savitzky-Golay, SNV or MSC were applied, the accuracy was 84, 85 and 87%, respectively.

The wavelengths selected by SPA algorithm coupled with LDA model were three (1082, 1385, 1461 nm) for smoothing pre-processing and eight (1144, 1272, 1396, 1490, 1516, 1546, 1575, 1603 nm) using smoothing associated with first derivative pre-processing, among the 228 available variables. The variables selected by SPA are spread throughout the spectrum in regions as the R-O-R stretching of aliphatic ethers (1070-1150 nm), the second overtone C-H of aromatic structure (1144 nm), the C-H combination (around 1395 nm), the first overtone N-H stretching of urea (1460 and 1490 nm), the first overtone N-H stretching of protein (around 1510 nm) and the first overtone C-H stretching (around 1570 nm) (Shenk, Workman Jr, & Westerhaus, 2001; Mistry, 2009). These results confirm that the myopathies are related to changes in protein and lipid content. In addition, it indicates that using three or eight important variables by SPA were efficient enough to discriminate the Normal, WS and WB (isolated or associated with WS) breast meat and constructed an optimal model with 92-93% correct classification.

The SIMCA model presented accuracy of 91, 89, 84, 73 and 80% on prediction set for smoothing, and smoothing associated with first and second derivatives, SNV and MSC pre-processing data, respectively. The results denote the lesser potentiality to identify the breasts groups compared to SPA-LDA model. In addition, Normal, WS and WB+WS/WB in SIMCA model obtained sensitivity (0.91, 0.89, 0.92) and specificity (0.93, 0.95, 0.98) for smoothing pre-processing data, and sensitivity (0.98, 0.78, 0.90) and specificity (0.86, 1.00, 0.99) for smoothing and first derivative.

The SPA-LDA model achieved high accuracy for smoothing, associated or not with the 1st derivative pre-processing data, demonstrating that these meats investigated can be identified using NIR spectroscopy with SPA-LDA algorithm. Previous studies involving the use of rapid and non-destructive techniques, observed a high accuracy on WB meat identification using Computer Vision System-CVS (91.8%) and NIR spectroscopy range of 1150-2150 nm (97.5%) (Geronimo et al., 2019).

Conclusion

This study demonstrated that chicken birds, even at a young age (4-5 weeks), are able to develop WS, WB and WS/WB myopathies, although with lower percentage of occurrence and presenting milder degrees of these myopathies compared to birds with more advanced slaughter age. In addition, NIRS combined with chemometric techniques can be used as a tool to discriminate among chicken breast according to presence or absence of WB and WS myopathies, regardless of the age at which birds are slaughtered. SPA-LDA algorithm applied to smoothing and smoothing with first derivative pre-processing data resulted the most appropriate approach. This technique has potential for application in industrial processing lines as it is a non-destructive method, in addition to being able to distinguish and separate the affected muscles according to their class in Normal, WS and WB+WS/WB, being an alternative to the conventional method of identification based on palpation and visual aspect of muscle. However, the use of this method was not efficient to differentiate WB and WS/WB muscles.

Acknowledgements

The authors acknowledge the CNPq – Brazilian National Council for Scientific and Technological Development (Process number 430832/2016-8).

Author Contributions

L.M. Carvalho collected test data, performed the multivariate data analysis and drafted the manuscript. M.S. Madruga conceived of the presented idea and supervised the project. M. Estévez contributed to the final version of the manuscript. A.T. Badaró supported data analysis and interpreted the results. D.F. Barbin helped shape the research and was in charge of overall direction and planning.

Reference

Brazilian Association of Animal Protein. (2019). *Annual report 2019*. http://www.brazilianchicken.com.br/files/publicacoes/31662c4161e289b4bca54c69cbf74fb1.pdf

Adabi, S.G., & Soncu, E.D. (2019). White striping prevalence and its effect on meat quality of broiler breast fillets under commercial conditions. *Journal of Animal Physiology and Animal Nutrition*, 103(4), 1060-1069. https://doi.org/10.1111/jpn.13092

Alander, J.T., Bochko, V., Martinkauppi, B., Sarawong, S., & Mantere, T. (2013). A review of optical nondestructive visual and near-infrared methods for food quality and safety. *International Journal of Spectroscopy*, 2013. https://doi.org/10.1155/2013/341402

Alnahhas, N., Berri, C., Chabault, M., Chartrin, P., Boulay, M., Bourin, M.C., & Bihan-Duval, E.L. (2016). 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*, 17, 61. https://doi.org/10.1186/s12863-016-0369-2

AOAC. (2000). *Official Methods of Analysis*. Gaithersburg, Maryland, USA: Association of Official Analytical Chemists. Methods 981.10, 950.46, 990.26.

Bailey, R.A., Watson, K.A., Bilgili, S.F., & Avendano, S. (2015). The genetic basis of pectoralis major myopathies in modern broiler chicken lines. *Poultry Science*, 94(12), 2870-2879. https://doi.org/10.3382/ps/pev304

Barbin, D.F., ElMasry, G., Sun, D-W., & Allen, P. (2013). Non-destructive determination of chemical composition in intact and minced pork using near-infrared hyperspectral imaging. *Food Chemistry*, 138(2-3), 1162-1171. https://doi.org/10.1016/j.foodchem.2012.11.120

Barbut, S., Sosnicki, A.A., Lonergan, S.M., Knapp, T., Ciobanu, D.C., Gatcliffe, L.J., … Wilson, E.W. (2008). Progress in reducing the pale, soft and exudative (PSE) problem in pork and poultry meat. *Meat science*, 79(1), 46-63. https://doi.org/10.1016/j.meatsci.2007.07.031

Dalle Zotte, A., Tasoniero, G., Russo, E., Longoni, C., & Cecchinato (2015). Impact of coccidiosis control program and feeding plan on white striping prevalence and severity degree on broiler breast fillets evaluated at three growing ages. *Poultry Science*, 94(9), 2114-2123. https://doi.org/10.3382/ps/pev205

Eldin, A.B. (2011). Near infra red spectroscopy. In: Akyar, I. (Eds.), *Wide spectra of quality control* (pp. 237-248). Rijeka, Croatia: IntechOpen. https://doi.org/10.5772/24208

FAOSTAT. (2019). *FAOSTAT database*. [Data set]. Download Data: Livestock Primary. http://www.fao.org/faostat/en/#data/QL

Folch, J., Lees, M., & Stanley, G.H.S. (1957). A Simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226(1), 497-509.

Geronimo, B. C., Mastelini, S. M., Carvalho, R. H., Barbon Júnior, S., Barbin, D. F., Shimokomaki, M., & Ida, E. I. (2018). Computer vision system and near-infrared spectroscopy for identification and classification of chicken with wooden breast, and physicochemical and technological characterization. *Infrared Physics & Technology*, 96, 303-310. https://doi.org/10.1016/j.infrared.2018.11.036

Kuttappan, V. A., Brewer, V. B., Clark, F. D., McKee, S. R., Meullenet, J. F., Emmert, J. L., & Owens, C. M. (2009). Effect of white striping on the histological and meat quality characteristics of broiler fillets. *Poultry Science*, 88(447), 136-137. (Abstr.)

Kuttappan, V. A., Brewer, V. B., Apple, J. K., Waldroup, P. W., & Owens, C. M. (2012). Influence of growth rate on the occurrence of white striping in broiler breast fillets. *Poultry Science*, 91(10), 2677-2685. https://doi.org/10.3382/ps.2012-02259

Kuttappan, V.A., Shivaprasad, H.L., Shaw, D.P., Valentine, B.A., Hagis, B.M., Clark, F.D., … & Owens, C.M. (2013). Pathological changes associated with white striping in broiler breast muscle. *Poultry Science*, 92(2), 331-338. https://doi.org/10.3382/ps.2012-02646

Lorenzi, M., Mudalal, S., Cavani, C., & Petracci, M. (2014). Incidence of white striping under commercial conditions in medium and heavy broiler chickens in Italy. *Journal of Applied Poultry Research*, 23(4), 754-758. https://doi.org/10.3382/japr.2014-00968

MacRae, V.E., Gilpin, S., Sandercock, D.A., Hunter, R.R., & Mitchell, M.A. (2007). A Comparison of breast muscle characteristics in three broiler great-grandparent lines. *Poultry Science*, 86(2), 382-385. https://doi.org/10.1093/ps/86.2.382

Malila, Y., U-chupaj, J., Srimarut, Y., Chaiwiwattrakul, P., Uengwetwanit, T., Arayamethakorn, S., … Visessanguan, W. (2018). Monitoring of white striping and wooden breast cases and impacts on quality of breast meat collected from commercial broilers

(Gallus gallus). *Asian-Australasian Journal of Animal Science*, 31(11), 1807-1817. https://doi.org/10.5713/ajas.18.0355

Marques, A.S., Moraes, E.P., Júnior, M.A.A., Moura, A.D., Neto, V.F.A., Neto, R.M., & Lima, K.M.G. (2015). Rapid discrimination of klebsiella pneumoniae carbapenemase 2 – producing and non-producing klebsiella pneumoniae strains using near-infrared spectroscopy (NIRS) and multivariate analysis. *Talanta*, 134, 126-131. https://doi.org/10.1016/j.talanta.2014.11.006

Mistry, B.D. (2009). Infrared spectroscopy. In: Mistry, B.D. (Eds), *A handbook of spectroscopic data chemistry (UV, IR, PMR, CNMR and mass spectroscopy)* (pp. 26-63). Jaipur, India: Oxford Book Company.

Mudalal, S., Babini, E., Cavani, C., & Petracci, M. (2014). Quantity and functionality of protein fractions in chicken breast fillets affected by white striping. *Poultry Science*, 93(8), 2108-2116. https://doi.org/10.3382/ps.2014-03911

Mutryn, M.F., Brannick, E.M., Fu, W., Lee, W.R., & Abasht, B. (2015). Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing. *BMC Genomics*, 16, 399. https://doi.org/10.1186/s12864-015-1623-0

Paxton, H., Anthony, N.B., Corr, S.A., & Hutchinson, J.R. (2010). The effects of A comparative study across modern and ancestral populations. *Journal of Anatomy*, 217(2), 153-166. https://doi.org/10.1111/j.1469-7580.2010.01251.x

Perez, I.M.N., Badaró, A.T., Barbon Jr, S., Barbon, A.P.A.C., Pollonio, M.A.R., & Barbin, D.F. (2018). Classification of chicken parts using a portable near-infrared (NIR) spectrophotometer and machine learning. *Applied Spectroscopy*, 72(12), 1774-1780. https://doi.org/10.1177/0003702818788878

Peng, Y., & Wang, W. (2015). Application of Near-infrared Spectroscopy for assessing meat quality and safety. In: Theophile, T. (Ed.), *Infrared Spectroscopy - Anharmonicity of Biomolecules*, *Crosslinking of Biopolymers* (pp.137-163). Rijeka, Croatia: IntechOpen. https://doi.org/10.5772/58912

Petracci, M., & Cavani, C. (2012). Muscle growth and poultry meat quality issues. *Nutrients*, 4(1), 1-12. https://doi.org/10.3390/nu4010001

Petracci, M., Mudalal, S., Bonfiglio, A., & Cavani, C. (2013). Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. *Poultry Science*, 92(6), 1670-1675. https://doi.org/10.3382/ps.2012-03001

Petracci, M., Mudalal, S., Babini, E., & Cavani. C. (2014). Effect of White striping on chemical composition and nutritional value of chicken breast meat. *Italian Journal of Animal Science*, 13(1), Article 3138. https://doi.org/10.4081/ijas.2014.3138

Petracci, M., Soglia, F., Madruga, M., Carvalho, L., Ida, E., & Estévez, M. (2019). Wooden-breast, white striping, and spaghetti meat: Causes, consequences and consumer perception of emerging broiler meat abnormalities. *Comprehensive Reviews in Food Science and Food Safe*ty, 18(2), 565-583. https://doi.org/10.1111/1541-4337.12431

Russo, E., Drigo, M., Longoni, C., Pezzotti, R., Fasoli, P., & Recordati, C. (2015). Evaluation of White Striping prevalence and predisposing factors in broilers at slaughter. *Poultry Science*, 94(8), 1843-1848. https://doi.org/10.3382/ps/pev172

Silva, C.S., Borba, F.S.L., Pimente, M.F., Pontes, M.J.C., Honorato, R.S., & Pasquini, C. (2013). Classification of blue pen ink using infrared spectroscopy and linear discriminant analysis. *Microchemical Journal*, 109, 122-127. https://doi.org/10.1016/j.microc.2012.03.025

Schmidt, C. J., Persia, M. E., Feierstein, E., Kingham, B., & Saylor, W.W. (2009). Comparison of a modern broiler line and a heritage line unselected since the 1950s. *Poultry Science*, 88(12), 2610-2619. https://doi.org/10.3382/ps.2009-00055

Shenk, J.S., Workman Jr., J.J., & Westerhaus, M.O. (2001). Application of NIR spectroscopy to agricultural products. In: Burns, D.A., & Ciurczak, E.W. (Eds.), *Handbook of Naer-Infrared Analysis* (pp. 419-474). New York, NY: Marcel Dekker Inc.

Sihvo, H. K., Immonen, K., & Poulanne, E. (2014). Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. *Veterinary Pathology*, 51(3), 619-623. https://doi.org/10.1177/0300985813497488

Sihvo, H. K., Lindén, J., Airas, N., Immonen, K., & Poulanne, E. (2016). A myodegenerative disease in the naturally hypertrophic pectoral muscle of broiler chickens: an animal model candidate. *Journal of Comparative Pathology*, 154(1), 60. https://doi.org/10.1016/j.jcpa.2015.10.007

Tickle, P.G., Paxton, H., Rankin, J.W., Hutchinson, J.R., & Codd, J.R. (2014). Anatomical and biomechanical traits of broiler chickens across ontogeny. Part I. Anatomy of the musculoskeletal respiratory apparatus and changes in organ size. *PeerJ*, 2, Article e432. https://doi.org/10.7717/peerj.432

Tijare, V.V., Yang, F.L., Kuttappan, V.A., Alvarado, C.Z., Coon, C.N., & Owens, C.M. (2016). Meat quality of broiler breast fillets with white striping and woody breast muscle myopathies. *Poultry Science*, 95(9), 2167-2173. https://doi.org/10.3382/ps/pew129

Trocino, A., Piccirillo, A., Birolo, M., Radaelli, G., Bertotto, D., Filiou, E., ... Xiccato, G. (2015). Effect of genotype, gender and feed restriction on growth, meat quality and the occurrence of white striping and wooden breast in broiler chickens. *Poultry Science*, 94(12), 2996-3004. https://doi.org/10.3382/ps/pev296

Velleman, S.G., & Clark, D.L. (2015). Histopathologic and myogenic gene expression changes associated with wooden breast in broiler breast muscles. *American Association of Avian Pathologists*, 59(3), 410-418. https://doi.org/10.1637/11097-042015-Reg.1

Wold, J.P., Veiseth-Kent, E., Host, V., & Lovland, A. (2017). Rapid on-line detection and grading of wooden breast myopathy in chicken fillets by near-infrared spectroscopy. *PLoS One*, 12(3), Article e0173384. https://doi.org/10.1371/journal.pone.0173384

Table 1 – Effect of breast myopathies on proximate composition of birds slaughtered at 6-7 weeks

Parameter	N	WS[2]	WB[3]	WS/WB[4]	p-Value
Moisture[1]	76.14±1.16	75.90±1.18	78.34±3.15	77.93±1.56	ns
Protein[1]	22.91±0.66[a]	20.31±0.92[b]	20.39±2.86[b]	20.20±2.00[b]	*
Lipid[1]	2.07±0.33[b]	3.20±0.59[a]	2.80±0.56[a]	3.21±0.38[a]	**
Collagen[1]	0.35±0.05[b]	0.48±0.03[a]	0.49±0.06[a]	0.53±0.04[a]	**

[a,b,c] Mean values within the same parameter followed by different superscript letters in the same row significantly differ by the Tukey test (*p<0.05; **: p<0.001; ns: no significant).

[1] Results expressed as g/100 muscle.

[2] Apparent stretch marks on the entire surface of the muscle (thickness > 1 mm).

[3] Muscle with extreme and extensive hardness on the entire surface.

[4] Muscle with extensive hardness and apparent stretch marks on the entire surface.

Table 2 – Results for SPA-LDA and SIMCA classification models for Normal, White striping and Wooden breast meats (three sample classes).

Models	Pre-treatment	Sample	Calibration				Prediction			
			Precision	Sensitivity	Specificity	Accuracy	Precision	Sensitivity	Specificity	Accuracy
SPA-LDA	Smooth	N	0.73	0.81	0.89	0.85	0.88	0.84	0.96	0.92
		WS	0.67	0.72	0.87		0.83	0.91	0.93	
		WB+WB/WS	0.92	0.83	0.94		0.95	0.93	0.95	
	Smooth and 1st der	N	0.71	0.77	0.89	0.83	0.89	0.87	0.96	0.93
		WS	0.67	0.70	0.88		0.81	0.96	0.92	
		WB+WB/WS	0.90	0.83	0.92		0.99	0.90	0.99	
	Smooth and 2nd der	N	0.64	0.71	0.86	0.78	0.71	0.80	0.88	0.84
		WS	0.60	0.62	0.85		0.66	0.78	0.85	
		WB+WB/WS	0.85	0.78	0.88		0.95	0.78	0.96	
	Smooth and SNV	N	0.72	0.69	0.90	0.80	0.97	0.56	0.99	0.85
		WS	0.62	0.65	0.86		0.61	0.96	0.78	
		WB+WB/WS	0.83	0.83	0.84		0.94	0.86	0.95	
	Smooth and MSC	N	0.72	0.69	0.90	0.80	0.94	0.60	0.99	0.87
		WS	0.64	0.74	0.86		0.69	0.98	0.84	
		WB+WB/WS	0.82	0.78	0.85		0.92	0.90	0.94	

SIMCA	Smooth	N	0.69	0.85	0.86	0.80	0.83	0.91	0.93	0.91
		WS	0.73	0.66	0.91		0.88	0.89	0.95	
		WB+WB/WS	0.92	0.85	0.93		0.98	0.92	0.98	
	Smooth and 1st der	N	0.57	0.91	0.75	0.73	0.71	0.98	0.86	0.89
		WS	0.90	0.28	0.99		1.00	0.78	1.00	
		WB+WB/WS	0.83	0.87	0.84		0.99	0.90	0.99	
	Smooth and 2nd der	N	0.48	0.96	0.63	0.69	0.63	1.00	0.78	0.84
		WS	0.94	0.37	0.99		1.00	0.67	1.00	
		WB+WB/WS	0.92	0.71	0.94		1.00	0.85	1.00	
	Smooth and SNV	N	0.37	0.98	0.40	0.51	0.61	0.93	0.78	0.73
		WS	0.75	0.32	0.96		0.71	0.87	0.87	
		WB+WB/WS	0.88	0.34	0.96		0.96	0.55	0.98	
	Smooth and MSC	N	0.36	0.98	0.38	0.50	0.60	0.98	0.76	0.80
		WS	0.79	0.25	0.98		0.98	0.76	0.99	
		WB+WB/WS	0.89	0.37	0.96		0.95	0.72	0.96	

Figure 1 – *Pectoralis major* muscle classification according to type and degree of myopathies.

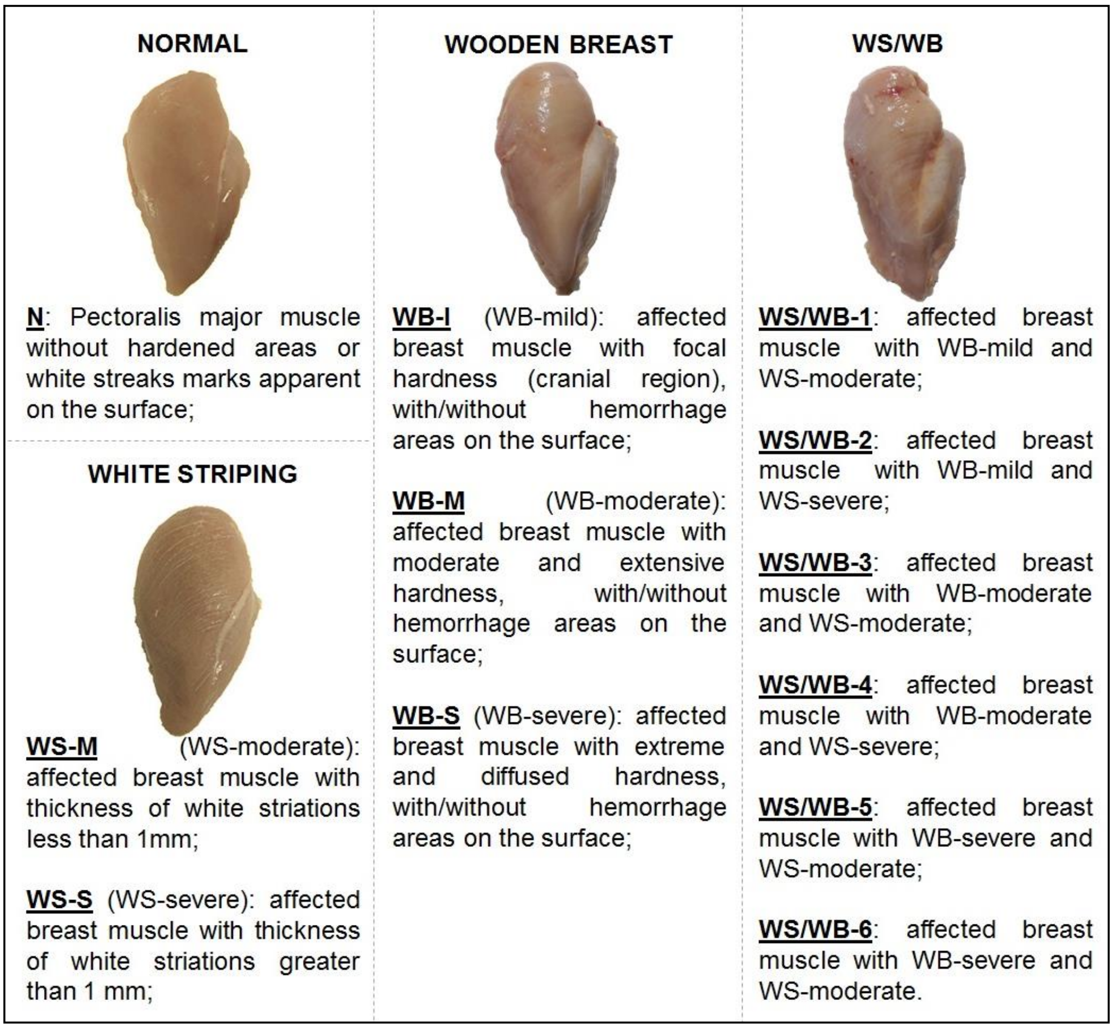
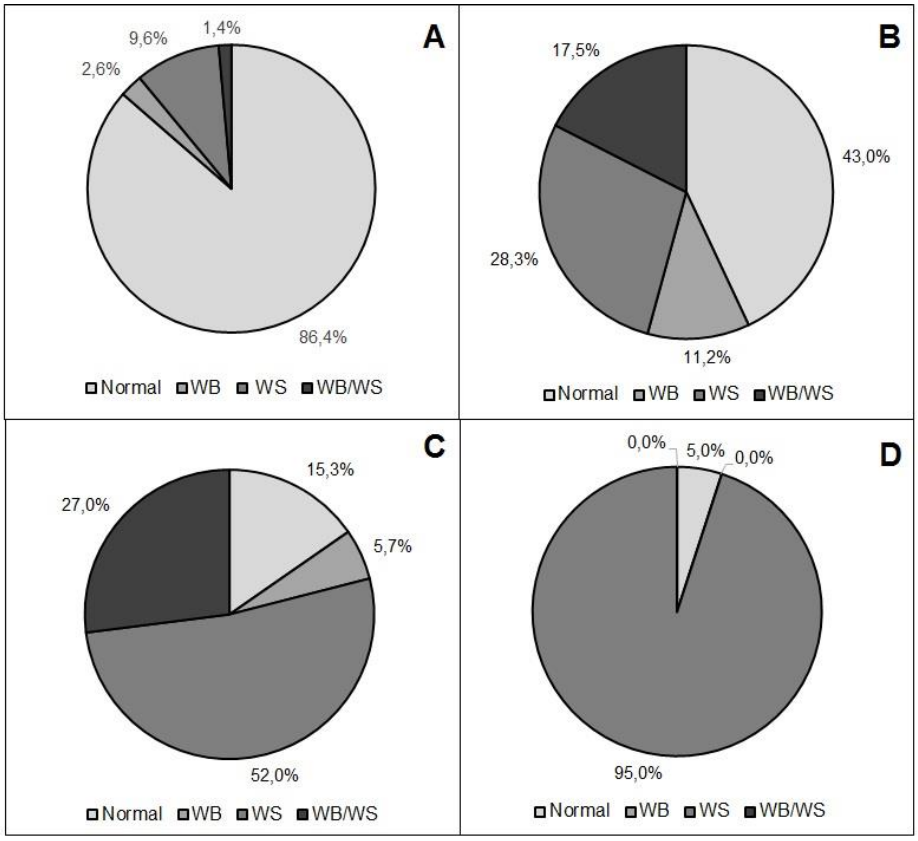
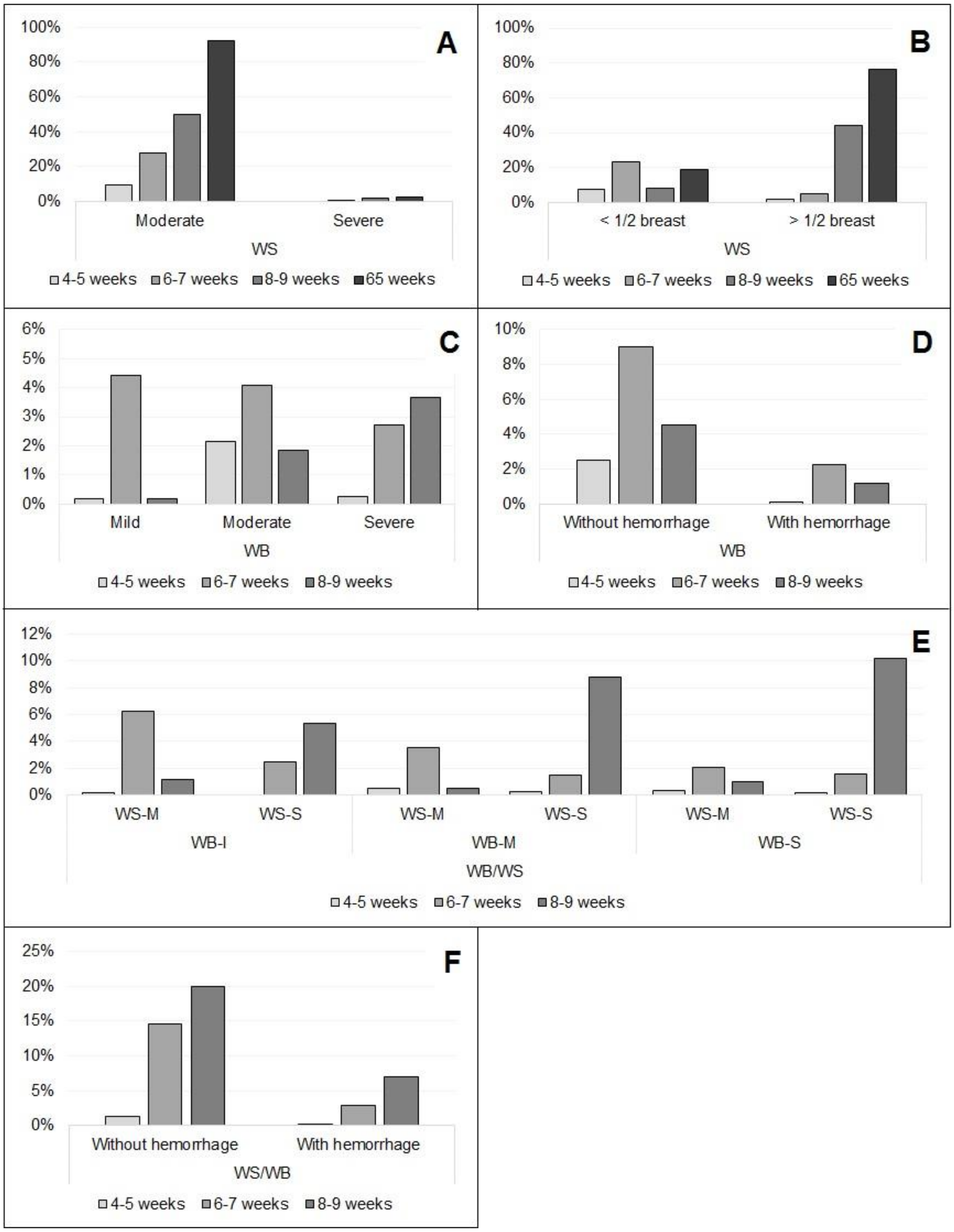


Figure 2 – The occurrence of isolated or combined WB and WS myopathies in breasts from birds slaughtered at different ages under commercial conditions



A: birds slaughtered at 4-5 weeks (G1; n= 1,200); B: birds slaughtered at 6-7 weeks (G2; n= 6,919); C: birds slaughtered at 8-9 weeks (G3; n= 600); D: birds slaughtered at 65 weeks (G4; n= 240).

Figure 3 – Categories description for breast meat based on myopathies scores.



A: Stretch mark thickness of breast with WS myopathy (moderate: < 1mm; severe: > 1 mm); B: Muscle areas affected by white stretch marks in breasts with WS (less or more than half of the muscle surface); C: Degree and extent of hardness in breasts with WB myopathy (mild: focal hardness; moderate: moderate and extensive hardness; severe: extreme and diffused hardness); D: Presence of hemorrhage on the surface of breasts with WB; E: Severity degree of combined WS and WB myopathies; F: Presence of hemorrhage on the surface of breasts with WS/WB.

Figure 4 – Raw mean NIR spectrum of breast meat.

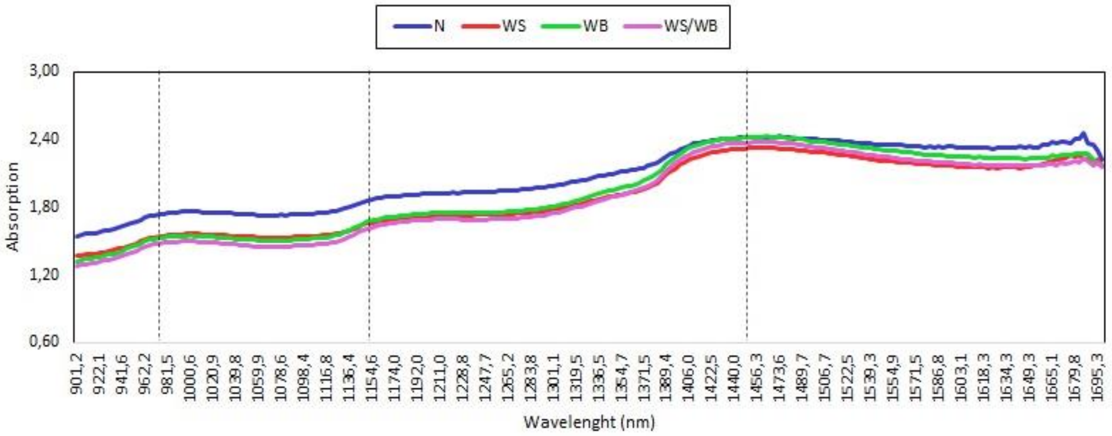
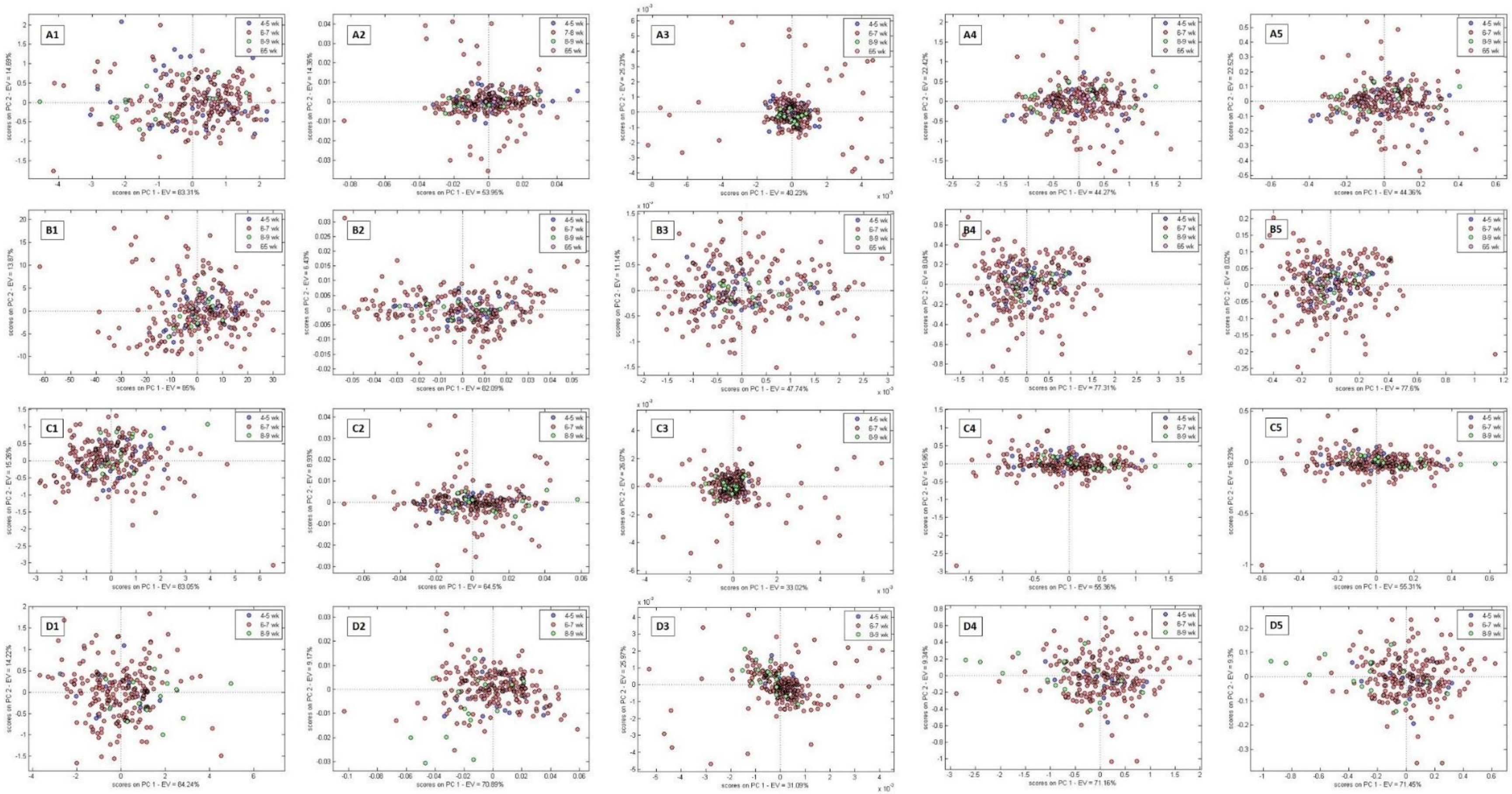
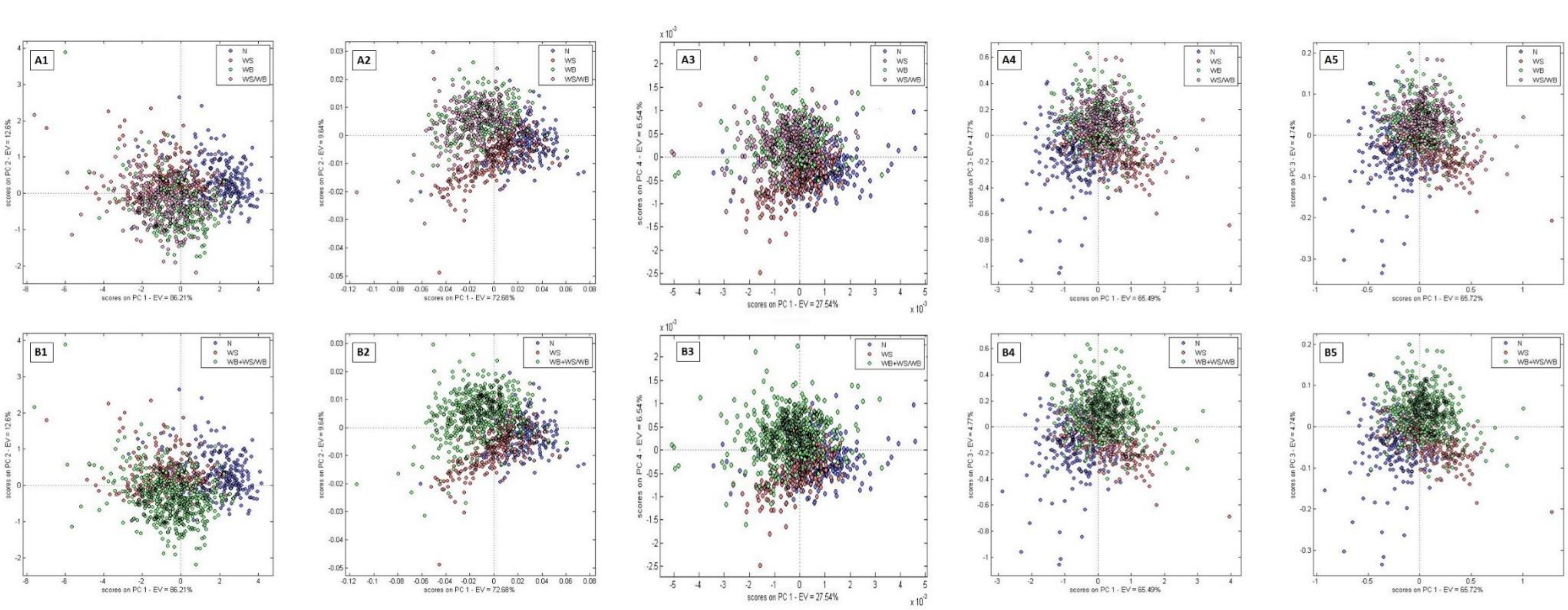


Figure 5 – PCA score of birds with different slaughtering age.



A: Normal; B: White striping; C: Wooden breast; D: WS/WB; 1: smoothing pretreatment; 2: smoothing and first derivative pretreatment; 3: smoothing and second derivative pretreatment; 4: smoothing and SNV pretreatment; 5: smoothing and MSC pretreatment.

Figure 6 – PCA score for Normal, WS and WB meats.



A: four sample classes (N, WS, WB, WS/WB); B: three sample classes (N, WS, WB+WS/WB); 1: smoothing pretreatment; 2: smoothing and first derivative pretreatment; 3: smoothing and second derivative pretreatment; 4: smoothing and SNV pretreatment; 5: smoothing and MSC pretreatment.

4.2 ARTIGO II – PINPOINTING OXIDATIVE STRESS BEHIND THE WHITE STRIPING MYOPATHY: DEPLETION OF ANTIOXIDANT DEFENSES AND ACCRETION OF OXIDIZED PROTEINS AND IMPAIRED PROTEOSTASIS

O artigo foi submetido ao periódico Food Science and Technology – LWT em 26 de maio de 2020, sob o título *Pinpointing oxidative stress behind the white striping myopathy: depletion of antioxidant defenses and accretion of oxidized proteins and impaired proteostasis* (ANEXO C).

Pinpointing oxidative stress behind the White Striping myopathy: Depletion of antioxidant defenses, accretion of oxidized proteins and impaired proteostasis

Leila M. Carvalho[1], Josué Delgado[2], Marta S. Madruga[1], Mario Estévez[3]

[1] Postgraduate program in Food Science and Technology. Department of Food Engineering, Federal University of Paraiba, João Pessoa, Paraíba, Brazil

[2] Heart Clinical Unit, Virgen de la Victoria University Clinic Hospital. Institute of Biomedical Research in Malaga. IBIMA. CIBERCV. University of Málaga, Málaga, Spain.

[2] Institute of Meat and Meat Products (IPROCAR), TECAL Research Group, University of Extremadura, Cáceres, Spain.

*Corresponding autor: Mario Estévez. Institute of Meat and Meat Products (IPROCAR), University of Extremadura, Avd. Universidad, sn. 10003, Cáceres, Spain. Phone number: +34 927251390. Email: mariovet@unex.es

Abstract

The study aimed to investigate the molecular mechanisms involved in the onset of the White Striping (WS) myopathy with particular attention to the role of oxidative stress and protein oxidation in the loss of meat quality. Commercial chicken breasts were classified as Normal (n=10), WS-M (moderate degree; white stripes < 1 mm thickness, n=10) and WS-S (severe degree; white stripes > 1 mm thickness, n=10). Samples were analyzed for physico-chemical properties and quality traits, endogenous antioxidant defenses, oxidative damage to lipids and proteins, and discriminating sarcoplasmic proteins by using a Q-Exactive Orbitrap equipment. WS breasts presented higher pH, hardness, redness, yellowness, lipid, protein and collagen content, and lower lightness and WHC compared to the Normal breast. Compared to the latter, WS-S had a more severe loss of protein thiols, reduced activity of antioxidant enzymes, and consequently, had greater accretion of malondialdehyde, allysine and Schiff base structures. The analysis of sarcoplasmic proteins revealed that muscles severely affected by the myopathy suffered a chronic impairment of physiological and metabolic processes and altered protein turnover plausibly mediated by oxidative stress and accumulation of oxidized proteins.

Keywords: white-striping; oxidative stress; protein oxidation; protein turnover; antioxidant enzymes.

1. Introduction

One third of the meat produced worldwide is poultry and chicken meat is the most preferred in countries such as USA, Australia, Brazil and Canada, among others (OECD, 2018). As poultry consumption has intensely trended higher over the last 40 years in USA, poultry meat production has concomitantly increased to peak more than 100 million tons per year (USDA, 2019). Fulfilling the worldwide demand for chicken meat has led to selecting broilers for fast growth and high carcass yields (Petracci et al. 2019). Such fast muscle hypertrophy has been identified as leading causes to the onset of assorted chicken breast myopathies (Petracci et al., 2019). Among these, white-striping (WS) is recognized as the one with the highest incidence and the most commonly found in supermarkets: 98% of birds at 9 weeks of age assessed in the US, displayed symptoms of the myopathy (Kuttappan et al., 2017). Breasts affected by the WS myopathy display impaired eating quality traits (i.e. appearance, texture), poor technological properties (i.e. water-holding), altered nutritional value, and may also affect consumer's acceptance and purchasing decision (Petracci et al., 2019)

As a reflection of the myodegeneration, the increased deposition of fat (lipidosis) and connective tissue (fibrosis), occurs along with interstitial inflammation, edema, infiltration of inflammatory cells and necrosis (Kuttappan et al., 2013). In a recent review, Petracci et al. (2019) summarized the potential underlying mechanisms behind the pathogenesis of WS and oxidative stress seems to play a central role. Yet, the fundamental mechanisms of such processes are not well understood. A better comprehension of the molecular basis of this myopathy may not only assist in the diagnosis and prevention of the disorder; it may enable a more efficient management of the meat from these animals to alleviate the symptoms and improve meat quality. Oxidative stress in muscle tissue involves an imbalance between pro-oxidant factors such as reactive oxygen species and the antioxidant defenses such as glutathione (GSH), tocopherols, and antioxidant enzymes (catalase, GSH-peroxidase, superoxide dismutase, among others) (Estévez, 2015). The oxidative damage to proteins is a typical feature in such pro-oxidative environments and protein oxidation is known to lead to altered physiological conditions and disease in animals and humans (Garcia-Garcia et al., 2012). In muscle foods, protein oxidation is known to affect protein functionality, meat texture, and nutritional value, and involves certain toxicity risks (Estévez & Xiong, 2019). Consistently, several authors found increased protein oxidation markers in chicken breasts affected by myopathies such as WS and wooden breast (WB) (Soglia et al., 2016), and by altered postmortem changes such as PSE (Carvalho et al., 2017). Yet, the molecular pathways involved as well as the mechanisms by which protein oxidation may play a role in the progress of the myopathy and the occurrence of the symptoms is mostly unknown.

The present study aims to contribute to a better comprehension of the molecular mechanisms behind the onset of oxidative stress in chicken muscles affected by the WS conditions and assess the potential consequences of such stress for meat quality and safety.

2. Material and methods

2.1. Samples, identification and classification

Collection of chicken breasts was carried out in randomly selected supermarkets in Cáceres (Spain). Samples were allocated to one of the following three groups based on the criteria described by Kuttappan et al. (2012): Normal ([N] did not show white striation on breast surface), WS-moderate ([WS-M] exhibited white striations with < 1 mm thickness), and WS-severe ([WS-S] exhibited white striations with > 1 mm thickness). Sixty chicken breasts (n=20 of each type) were collected and used for the present study.

2.2. Physico-chemical composition

2.2.1. Chemical composition

Protein, moisture, ash and collagen contents were analyzed in Normal, WS-moderate and WS-severe meats, according to the Association of Official Analytical Chemists (AOAC, 2000). The fat content was determined according to the methodology described by Folch et al. (1957).

2.2.2. Physical properties

pH was determined by direct electrode insertion on the cranial surface of each *Pectoralis major* muscle using a meat pH meter system (HI 99163, Hanna Instruments, Woonsocket, Rhode Island, USA). Color was measured in three different areas at the dorsal surface of the breast muscle using a Minolta colorimeter (Chroma Meter CR-300, Minolta Co., Osaka, Japan) in the CIELAB system (L*=lightness; a*=redness, and b*=yellowness) according to Carvalho et al. (2017). Water holding capacity (WHC) was measured as percentage of cooking loss as described by Carvalho et al. (2017). Hardness was performed in raw meat samples cut into parallelepiped (perpendicular to the muscle surface) with dimensions of 25 x 25 x 10 mm (length x width x thickness, respectively) and analyzed on a texturometer (TA.TXplus texturometer, Stable Micro Systems, Godalming, Surrey, UK). The samples were compressed twice to 50% of their original height with compression flat cylindrical aluminum probe (50 mm diameter) at a test-speed of 50 mm/min. The results were expressed in Newtons (N).

2.3.Oxidative damage

2.3.1. TBARS

Lipid oxidation was determined in breast meat by the thiobarbituric acid-reactive substances (TBA-RS) assay using the method of Ganhão, Estévez & Morcuende (2011). Briefly, 2.5 g of each breast meat were homogenized with 7.5 mL of 3.86% perchloric acid, using an ultraturrax. The homogenate was centrifuged (4500 g for 3 min) and filtered through double filter paper. Two mL of the filtrate were mixed with 2 mL of 0.02 mol L^{-1} TBA in perchloric acid (3.86%) in test tubes (duplicate). The test tubes were homogenized by vortex and incubated in a water bath (90-100°C) for 30 min, in order to develop the color reaction. All tubes test were centrifuged (4500 g for 2 min) and the absorbance was measured at 532 nm using a spectrophotometer (Shimadzu UV-1800, Japan) against a blank containing 2 mL of 3.86% perchloric acid and 2 mL of TBA reagent. The results from the samples were plotted against a standard curve prepared with known concentrations of tetraethoxypropane (TEP). The results were expressed as mg malondialdehyde (MDA) kg^{-1} breast meat.

2.3.3. Allysine

Allysine, a major lysine oxidation product and marker of oxidative stress, was quantified by following the procedure described by Utrera, Morcuende, Rodríguez-Carpena, & Estévez (2011). Briefly, chicken breast samples (1.0 g) were derivatized with 0.5 mL of 50 mmol/L aminobenzoic acid (ABA) and subsequently hydrolyzed with 1.0 mL of 6 mol/L HCl. Hydrolysates were dried in vacuo, reconstituted in Milli-Q water and filtered through a Polyvinylidene difluoride (PVDF) syringe filter (0.45 μm pore size, Pall Corp., New York, USA). Injection was performed in a HPLC using a Cosmosil (Phenomenex, Torrance, California, USA) C18-AR-II RP-HPLC column (5 μm, 150 × 4.6 mm) and a guard column (10 × 4.6 mm) filled with the same material. The Shimadzu "Prominence" HPLC apparatus (Shimadzu Corp., Kyoto, Japan) was equiped with a quaternary solvent delivery system (LC-20 AD), a DGU-20AS online degasser, an SIL-20A autosampler, an RF-10A XL fluorescence detector, and a CBM-20A system controller. 50 mmol/L sodium acetate buffer (pH 5.4, eluent A) and acetonitrile (ACN, eluent B) were used as eluents. A low-pressure gradient program was used, varying eluent B concentration from 0% (min 0) to 8% (min 20). The injection volume was 1 μL, the flow rate was kept at 1 mL/min, and the temperature of the column was maintained constant at 30 °C. Excitation and emission wavelengths were set at 283 nm and 350 nm, respectively. Identification of the derivatized semialdehydes in the Fluorescence Detector (FLD) chromatograms was carried out by comparing their retention times with those from a standard compound injected and analyzed under the above-mentioned conditions (Utrera et al., 2011). The peak corresponding to allysine-ABA was manually integrated from FLD chromatograms and resulting areas plotted against an ABA standard curve (ranging from 0.1 to 0.5 mmol/L). Regression coefficients of >0.99 were obtained. The estimation of the quantities of allysine-ABA through an ABA standard curve was accomplished by assuming that the fluorescence emitted by 1 mol of ABA is equivalent to that emitted by 1 mol of the derivatized protein carbonyls. Results were expressed as nmol of allysine per mg of protein.

2.3.4. Protein cross-links

Protein cross-linking was assessed by means of Schiff bases fluorescence and quantification of disulphide bonds. The analysis of fluorescent Schiff bases was performed using fluorescence spectroscopy as described by Chelh, Gatellier & Santé-Lhoutellier (2007). Sample homogenates (1:10 w/v) in sodium phosphate buffer pH 6.0 with 8 M urea were obtained using an ultraturrax. After dilution (1:20 v/v), samples were transferred to a 4 mL quartz cuvette with four flat walls (101-QS 10 × 10 mm, Hellma Analytics, Müllheim, Germany). The emission spectrum for the Schiff base was recorded between 400 nm and 500 nm wavelength with excitation set at 350 nm (Perkin-Elmer LS 55 Luminescence spectrometer, Beaconsfield, UK). The excitation and emission slit widths were set at 10 nm and data were collected at 500 nm per minute. The results were expressed as units of fluorescence intensity emitted by Schiff base structures at 450 nm. These values were corrected according to the protein concentration of each sample by applying a correction factor (Cf = Pt/Pp) where Pt is the total average of the amount of protein from all samples and Pp is the content of protein in each type of sample.

Disulphide bonds were analyzed following the methodology of Rysman et al. (2014). One g of each sample was homogenized with the aid of ultraturrax with 25 mL of 100 mM tris buffer pH 8.0 added 6 M Guanidine Hydrochloride (GuHCl). Homogenates were followed for centrifugation (20 min at 1500 g and 4 °C), and the supernatants were filtered (qualitative filter paper, 11 μm particle retention). Three mL of filtrate were subjected to disulphide reduction by addition of 50 μL of 1-octanol and 100 μL of freshly prepared 30% (w/v) sodium borohydride in 1 M NaOH. After incubation at 50 °C for 30 min, an aliquot of 1.35 mL of 6 M HCl was added, followed by stirring for 10 min. Total thiols were determined with 4,4′-dithiodipyridine (4-DPS) in the reduced filtrates. A volume of 250 μL of the filtrate was mixed with 1.25 mL of 1 M citric acid buffer and pH 4.5 added 6 M GuHCl and 250 μL of 4-DPS solution (4 mM DPS-4 in 12 mM HCl). The absorbance was measured at 324 nm against 1 M citric acid buffer pH 4.5 added with 6 M GuHCl prior to the addition of 4-DPS (Apre) and after 30 min of reaction with 4-DPS in the light coat and at room temperature (Apos). A mixture of 1.25 mL of 1 M citric acid buffer pH 4.5 added of 6 M GuHCl and 250 μL of the 4-DPS solution was prepared as a white (Ablank). The absorbance corresponding to the thiol concentration was calculated by subtracting Apre and Ablank to Apos. 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). The disulphide content was calculated as half of the difference between total and free thiols divided by two.

2.4. *In vitro* activity of antioxidant enzymes

2.4.1. Extraction for antioxidant enzymes' activity

Extraction was performed in breast meat following the method of Carvalho et al. (2017) with minor modifications. The extractions were performed twice with one replicate. The muscle (5 g) was mixed with 35 mL of ice-cold phosphate buffer (extraction solvent, pH 7.0, 50 mM; disodium phosphate heptahydrate (Na_2HPO_4 x $7H_2O$) and KH_2PO_4). Samples were homogenized by ultraturrax (12000 rpm, ca. 45 s). After centrifugation (4500 g, 40 min, 4 °C), the supernatants were recovered and filtered over glass wool. These muscle extracts were used for the enzyme measurements of catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD).

2.4.2. Catalase (CAT)

CAT activity was measured according to the method described by Carvalho et al. (2017), with minor modifications. The aliquot of 100 µL extract of breast meat (kept in the ice-bath) were brought in a cuvette (1 cm path length), and 2.90 mL of H2O2 were added. Immediately, the absorbance was monitored at 240 nm during 180 seconds using a spectrophotometer (Shimadzu UV-1800, Japan). CAT activity was expressed in µmol × min-1 × g-1 (U/g). One unit (U) of CAT activity was defined as the amount of extract needed to decompose l µol of H_2O_2 per min. CAT activity was expressed as µmol x min-1 x g-1 (U/g).

2.4.3. Glutathione peroxidase (GSH-Px)

GSH-Px activity was determined in breast meat using the method described by Carvalho et al. (2017), with minor modifications. The aliquot 600 µL of the muscle extracts was mixed with 2.35 mL of reaction solvent containing 1.13 mM reduced glutathione, 0.57 mM EDTA, 1.13 mM NaN_3 and 1.7 units glutathione reductase in 100 mL phosphate buffer (pH 7.0, 50 mM; disodium phosphate heptahydrate ($Na_2HPO_4.7H_2O$) and KH_2PO_4 in MiliQ water). Twenty-six µL NADPH solution (17.3 mM), and 20 µL H_2O_2 solution were dispensed in cuvettes (1 cm path length). The absorbance was monitored at 340 nm during 600 seconds using a spectrophotometer (Shimadzu UV-1800). The extinction coefficient of 6.220 µL µmol-1 cm-1 for NADPH at 340 nm and 25 °C was used for the calculation. GSH-Px activity was expressed as µmol of oxidized NADPH µL-1 min-1 g-1 (U/g).

2.4.4. Superoxide Dismutase (SOD)

SOD activity was measured according to the procedure of Marklund & Marklund (1974) using inhibition of pyrogallol autoxidation in a basic medium. The supernatant fraction of the muscle homogenate was also used for the determination of SOD enzyme activity, 50 µl of pyrogallol (10 mM) were added to 2.9 ml of Tris–cacodylic buffer (pH =8.2, 50 mM with diethylenetriaminepentaacetic acid, DTPA). The rate of pyrogallol autoxidation in presence of 50 µl of muscle extract was compared to a blank (with 50 µl of buffer) by measuring the increase of absorbance at 420 nm during 360 seconds using a spectrophotometer (Shimadzu UV-1800). One unity was taken as the activity that inhibits the pyrogallol autoxidation by 50%. SOD activity was expressed as U/g of sample.

2.5. Proteomic approach

Protein extraction. Sarcoplasmic proteins fractions were extracted according to Zhu et al. (2011) with some alterations. 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 1200g 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 pallet was resuspended in 80 mL of some 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. The diluted SP extract was mixed with 250 µL 50% TCA, incubated on ice for 30 min and centrifuged at 1500 g for 10 min at 4 °C. The supernatant was discarded, the pellet was then homogenized in 500 µL of acetone (-20 °C), incubated at -20 °C for 1 h and centrifuged at the same setting. This procedure was repeated more than twice. The pellets were dried at room temperature, reconstituted in 1 mL of buffer urea (6 M urea, 2 M thiourea, 0.1 M Tris-HCl, pH 8) and filtered through a cellulose acetate membrane syringe filter (0.22 µm pore size, Pall Corp., New York, USA) and protein content was again determined using Bradford method. This solution was diluted to a final protein concentration of 3.33 mg/mL (FPS).

Label-free quantitative proteomic analyses. The preparation of samples for proteomic analyses was carried out as reported before by Delgado et al. (2019). Briefly, following dithiothreitol reduction and iodoacetamide-mediated alkylation, sequencing-grade trypsin (Promega, USA) and ProteaseMAX surfactant (Promega) were added and incubated overnight at 37 °C. Digested samples were desalted prior to analysis using ZipTip® C18 Tips (Merck, Germany). A Q-Exactive Plus mass spectrometer coupled to a Dionex Ultimate 3000 RSLCnano (Thermo Scientific) analyzed 0.75 µg from each digest. LC gradients ran from 5 to 45% B (A: 3% acetonitrile, 0.1% formic acid (FA), B: 80% acetonitrile, 0.1% FA) over 2 h on an EASY-Spray column, 50 cm×75 µm ID, PepMap RSLC C18, 2 μm (Thermo Scientific, CA, USA), and data was collected using a Top15 method for MS/MS scans (Delgado et al., 2019). Comparative proteome abundance and data analysis was carried out by using MaxQuant software (Version 1.6.0.13; www. maxquant.org/downloads.htm)(Cox & Mann, 2008), and Perseus (Version 1.6.0.7) 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, www.uniprot.org). The maximum peptide/protein false discovery rates (FDR) were set to 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 two replicates from at least one treatment. Quantitative analysis was performed using a t-test to compare treatments to the control. Only proteins with a fold change≥2 ($p < 0.05$) were included in the quantitative results (Delgado et al., 2019). 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.

2.6 Statistical analysis

Data from physical-chemical composition, oxidative damage and antioxidant enzymes' activity were analyzed by ANOVA using the different breast (Normal, WS-M, WS-S) as main factor.

3. Results and Discussion

3.1. Physico-chemical composition

The results from the physico-chemical properties of chicken breasts are shown in Table 1. Overall, the occurrence of the myopathy in the *Pectoralis major* muscle significantly influenced all parameters, except moisture content ($p>0.05$). The protein content was significantly higher in Normal chicken breast compared to WS-S (21.7 and 20.3 g/100g, respectively; $p<0.05$), while WS-M (20.7 g/100g) showed intermediate levels of protein. These results showed that the increased severity of WS meat implied a decreasing protein content, with this result being similar to that observed by Kuttappan et al. (2012). The lower protein content in breast meats affected by WS can be explained by the histopathological changes occurred during myodegeneration such as floccular/vacuolar degeneration and lysis of fibers (Kuttappan et al., 2013). It is known that the onset of the WS myopathy leads to loss of sarcoplasmic and contractile proteins, and changes in the amino acid profile, especially histidine, arginine and tryptophan (Adabi & Soncu, 2019). Along with the protein decrease, a significant increase in the lipid content was found in breasts affected by WS (N: 2.6 vs WS-M: 4.0 vs WS-S: 4.1 g/100g; $p < 0.001$). These results are consistent with Kuttappan et al. (2012), Soglia et al. (2016) and Adabi & Soncu (2019), among others. The WS breasts had a greater collagen ($p<0.01$) content compared to the Normal counterparts. The collagen content varied from 0.38 to 0.49 g/100g in Normal and WS-S chicken breasts, respectively. This same feature was also observed by Mudalal et al. (2014) and Petracci et al. (2014). According to Kuttappan (2013), the WS condition is characterized by degenerative lesions with replacement of muscle tissue chronically damaged with adipocytes (lipidosis) and connective tissue (fibrosis). These two histopathological changes would explain the higher lipid and collagen contents found in striped chicken meats. Chicken breasts with moderate and severe degrees of WS had higher pH value compared to Normal meat (Table 1). High pH values in WS breast muscles (~ 6) were also reported by Petracci et al. (2013), Mudalal et al. (2015) and Brambila, Bowker & Zhuang (2016). According to Mudalal et al. (2015), a high ultimate pH in muscles affected by the WS myopathy may be related to reduced glycogen content or impaired post-mortem acidification process. The water-holding capacity (WHC) as measured by retained water after cooking, was significantly lower (63.2 %) in WS-S than in the Normal chicken breasts (71.2 %) (Table 1). Once again, WS-M samples displayed intermediate values. These results were expected as chicken breasts muscles affected by WS and other myopathies have been found to display impaired functional properties (Petracci et al., 2019). Yet, the results are not consistent with the pH values as it is common knowledge that final pH value is positively correlated to WHC in postmortem muscles (Huff-Lonergan & Lonergan, 2005). These results indicate that in WS muscles, the effect of final pH on WHC is irrelevant and other factors may be more influential. The decrease in protein concentration and the severe conformational changes in muscle structure (lipidosis and fibrosis) may contribute to explaining the impaired WHC in WS breast muscles. Furthermore, proteins from WS muscles have been found to be severely affected by hydrolytic and oxidative changes during aging and subsequent storage (Soglia et al., 2016). Our hypothesis proposes that the oxidative damage to meat proteins from breast affected by the WS myopathy accounts for more of the impaired functionality displayed by these samples. Data discussed in the following section, showing a more severe protein oxidation in WS samples than in the Normal counterparts, provide strengthen to this proposal.

Color measurements in chicken breasts revealed significantly differences between Normal and WS groups (Table 1). The lightness (L* value) was higher in the Normal breast muscle compared to that affected by the WB myopathy ($p < 0.05$). Conversely, redness was higher in the WS meats compared to Normal meat ($p<0.05$). The b* values (yellowness) was significantly higher in WS-S than in Normal breast ($p<0.01$). These results are in agreement with Petracci et al. (2013) who reported that increasing severity of WS implied a more redness and yellowness on muscle surface. Previously, Kuttappan et al. (2017) noted a significant increase in b* parameter on the carcass from birds with 9 weeks. The higher yellowness could be related to the fat accumulation (lipidosis) observed in WS meats (Mudalal et al., 2014; Petracci et al., 2014). These altered color parameters may be the reflection of the impaired appearance of these abnormal chicken breasts.

Regarding to hardness, the three meats differed significantly from each other ($p < 0.05$). The WS-M resulted in higher hardness value compared with WS-S and Normal raw samples (98.1, 71.8, and 63.5 N, respectively). Similar behavior was observed by Brambila et al. (2016) who found significant differences in the intensity scores for hardness attribute between WS-S and Normal meats. This result may be explained by increased fibrosis on the damaged tissue (Kuttappan et al., 2013), and greater collagen content observed in WS chicken breast. Yet, a reduction of hardness in WS-S samples as compared to the WS-M counterparts was found and this outcome cannot be explained by differences in terms of lipid and collagen contents. A more likely explanation for the differences in texture between these samples would involve the onset of intense protein oxidative reactions. The results discussed in the following section, proving such intense protein decomposition, support the hypothesis that texture measurements in WS-M and WS-S samples were mostly affected by the degree of protein oxidation.

3.2.Oxidative damage

Results for the oxidative damage markers measured in chicken breasts affected by increasing degrees of WS myopathy are presented in Table 2. In general, breasts with a higher degree of myopathy had a significantly more intense loss of protein thiols and a significantly higher accretion of lipid and protein oxidation products (malondialdehyde, allysine and Schiff bases). Overall, these results illustrate that, as anticipated by other authors such as Petracci et al. (2019), oxidative stress may be a crucial underlying cause in the onset of the myopathy.

The TBARS index in raw samples showed a higher extent of lipid oxidation in WS-S (0.64 mg MDA/kg muscle), followed by WS-M (0.37 mg MDA/kg muscle), and finally, by the Normal breast (0.22 mg MDA/kg muscle). Some authors found similar results and attributed the outcome to the higher lipid content, and higher concentration of PUFA in WS chicken breast than in the Normal ones (Adabi & Soncu, 2019). Conversely, Soglia et al. (2016) did not observed an increase of TBARS values in WS meat. These increased rates of lipid oxidation in WS chicken breasts may not be a threat in terms of quality as the levels are below thresholds for detectable rancid flavor (Alfaia et al., 2010). Yet, these TBARS numbers indicate that the impaired physiological processed occurred during the onset of this myopathy may have promoted oxidative reactions and/or increased the susceptibility of muscle lipids to undergo oxidative degradation during further storage and processing. The interconnection between all these mechanisms will be discussed in due course.

Protein oxidation occur in the absence of oxidized lipids; but whenever lipids and proteins are co-oxidized in a complex food (such as meat systems), the transfer of oxidizing species between both biomolecules is plausible (Estévez, 2011). For instance, free radicals and hydroperoxides generated in the onset of lipid oxidation may affect neighboring proteins and initiate an oxidative chain reaction similar to lipid oxidation

(Estévez, 2011). The oxidative damage to proteins leads to various chemical modification and in the present study, the loss of free thiols was assessed together with the accumulation of assorted protein oxidation products. Consistent to the TBARS numbers, the loss of protein thiols was more intense in chicken breasts affected by the myopathy, but in this case, only the WS-S suffered such loss to a remarkable and significant extent ($p < 0.001$). The severe degree of WS led to a depletion of 70.7 % of the thiol concentration found in Normal meat. These results showed the remarkable severity of oxidative stress undergone by proteins from the WS-S samples as protein thiols are susceptible to ROS, and its depletion is a reflection of the oxidative degradation of sulphur amino acids (SAA) such as methionine and cysteine (Estévez et al., 2020). As a result of the oxidation of SAA by ROS, thiyl radicals ($S^{\bullet}$) are formed. Two $S^{\bullet}$ can react with each other to form a disulfide bound (protein cross-link) or intermediate unstable products can eventually be degraded to final oxidation products such as methionine sulphoxide (Estévez et al., 2020). Beyond the indication of oxidative stress, the loss of thiols has been linked to altered quality traits in muscle foods and the formation of potentially toxic compounds (Estévez & Xiong, 2019).

Allysine is a specific oxidation product from lysine, the most abundant protein carbonyl in biological systems and a reliable indicator of meat protein carbonylation (Estévez, 2011). An increase of allysine concentration was found in chicken breasts as the degree of WS increased from normal to severe (1.99 vs 2.29 vs 3.15 nmol allysine/ mg protein; $p < 0.01$). This represents an increase in allysine content of 15.1% (WS-M) and 58.3% (WS-S) compared to normal meat. In consistency with thiol oxidation, the formation of allysine was only significantly higher in the severe degree of the myopathy as compared to Normal samples. The carbonylation of proteins results in irreversible modification of essential amino acids, the loss of meat nutritional quality, and may also be implicated in pathogenesis of gastrointestinal disorders (Estévez & Xiong, 2019).

The formation of protein cross-links via formation of Schiff bases or disulphide bonds is other common and remarkable expression of the oxidative damage to proteins (Estévez et al., 2020). Chicken breasts affected by WS had significantly higher values (70.9 and 150%, respectively) of Schiff bases than Normal poultry breasts (Table 2). Chelh et al. (2007) pointed out that the Schiff bases are generated by interaction between aldehydic products of lipid oxidation and protein amino groups. Utrera et al. (2012) also highlight out that the allysine (specific lysine oxidation product) may be involved in cross-links with side chain of proteins amino groups by Schiff base formation. These observations are consistent with the current study as intense allysine and Schiff base formation concurred in the same samples plausibly explaining the implication of protein carbonyls in Schiff base formation. The study of disulphide bonds was divergent as WS-M breasts presented higher disulphide bonds compared to Normal meat and WS-S had, for this measurement, the lowest values (Table 2). As aforementioned, it is known that protein thiols may not only lead to the formation of disulfide bonds as some irreversible final oxidation products may also be formed (Estévez et al., 2020). The present results indicate that the severity degree of the myopathy strongly affected the fate of protein thiols. The oxidation of protein thiols in WS-M was limited and data suggest that most of the oxidized cysteine led to the formation of disulphide bonds. Conversely, a more severe oxidative stress in WS-S led to an intense depletion of thiols that plausibly turned into irreversible final oxidation products. The present results also clearly indicate that the toughness of WS-M chicken breasts was very likely caused by the formation of disulphide bonds while Schiff bases and other mechanisms of meat toughness (accumulation of collagen) played a negligible role. Rysman et al. (2014) reported that the formation of disulfide bonds formation on myosin tail region may cause a reduction in meat tenderness. These crosslinks may also affect the recognition sites for proteases, resulting in reduced digestibility (Rysman et al., 2014).

According to Petracci et al. (2019), the white striping myopathy is characterized by the degenerative muscle injury and inflammatory cell process. These lesions on muscle may explain a higher susceptibility of meat proteins from WS chicken breasts to oxidative degradation, since ROS are indispensables executors and modulators of degenerative-inflammatory phase during skeletal muscle regeneration (Kosakowska et al., 2015). The present study shows that proteins are major recipients of the oxidative damage to WS chicken breasts with consequences on meat quality.

3.3. Antioxidant defenses

The activity of the main skeletal muscle antioxidant enzymes was assessed in Normal chicken breast and in those affected by the WS myopathy (Table 3). The impact of the WS condition on the activities of endogenous antioxidant enzymes depended on the severity degree. Catalase (CAT), Glutathione peroxidase (GPX) and Superoxide dismutase (SOD) enzymes activities were significantly lower in WS-S compared to other samples. This decrease in activity variated from 41 to 48% (CAT), 61 to 75% (GPX) and 23 to 25% (SOD) compared to Normal and WS-moderate, respectively. WS-M samples tended to display higher enzyme activities than Normal chicken breasts and this difference was statistically significant for the GPX activity. CAT, GPX and SOD are endogenous antioxidant enzymes that contribute to minimize the damaging effects of ROS on muscle tissue (Carvalho et al., 2017). SOD function is to scavenge superoxide radical in the cell, while CAT, and GPX play an important role in the reduction of hydrogen peroxide to H_2O (Muller et al., 2007). It is known that meat affected by WS and other muscle growth abnormalities are more susceptible to oxidation, and that oxidative stress occurs during the onset of the myopathy (Petracci et al., 2019). The present results indicate that muscles affected by a moderate degree of the myopathy tended to have more intense activities of CAT and SOD and displayed higher GPX activities. It is plausible that WS-M muscles attempted to counteract the oxidative damage caused by ROS in muscle cells by promoting the expression and/or activity of the endogenous antioxidant enzymes. Taking into account the results from lipid and protein oxidation markers, this attempt failed as WS-M samples had higher concentration of TBARS, Schiff bases and disulphide bonds than Normal chicken samples. On the other hand, a higher degree of severity led to a collapse of the endogenous antioxidant enzymes, remarkable depletion of thiols and significant increases in TBARS, allysine and Schiff bases. The different response of WS-M and WS-S to the oxidative threat may also explain why the former had higher concentration of disulphide bonds than the latter despite of showing more resources against oxidative stress. The formation of disulphide bonds is compatible in a scenario of struggle against oxidative stress: thiols are oxidized to protect other valuable amino acid residues and therefore, reversible disulphide bonds are formed. These structures can be formed as a result of controlled physiological mechanisms of antioxidant protection and upon recovery of the oxidative attack, disulphide bonds can be reduced to yield the original thiols (Estévez et al., 2020). While this could have happened in the WS-M muscle, in the WS-S counterparts, a severe oxidative stress caused an intense depletion of antioxidant resources, failure of the antioxidant enzymes and irreversible thiol oxidation. While the precise reactions involved are not studied here, it is obvious that thiols from WS-S samples were oxidized into products different from disulphide bonds, such as methionine sulphoxide (Estévez et al., 2020).

3.4. Proteomics

Muscle proteins were extracted from Normal and WS-S chicken muscles under study and analyzed in order to gain further insight into the molecular mechanisms behind the onset of the myopathies. Among those, only discriminating proteins between Normal and WS-S chicken breasts are displayed in Table 4. The overall results and particularly, the discriminating proteins have been previously identified as relevant in the functionality of skeletal muscle. The proteasome reported by Doherty et al. (2004) in chicken breast by using MALDI-TOF is consistent with the current results. Yet, some of the discriminating proteins found in the present study enables expanding the existing knowledge on the underlying causes of chicken breast myopathies. According to our findings, the WS conditions altered the proteasome of chicken breasts. Discriminating proteins corroborate that an impaired muscle growth, altered metabolism and the onset of oxidative stress are behind the occurrence of this myopathy (Kuttappan et al., 2017). The downregulation of three enzymes involved in carbohydrate metabolism and energy supply such as, pyruvate kinase, creatine kinase and L-lactate dehydrogenase, are indicative of muscle stress and cellular damage (Brancaccio et al. 2010). In agreement with the present results, Meloche et al. (2018) found alterations in the abundance of these enzymes in chicken muscles affected by myopathies. Zambonelli et al. (2017) also found significantly lower abundance of pyruvate kinase and creatine kinase in muscles affected by WS myopathy than in normal muscles. Other group of discriminating proteins such as sarcoplasmic reticulum Ca^{2+} ATPase, sarcalumenin and calsequestrin-2 are implicated in calcium metabolism and muscle contraction. The up-regulation of these proteins could be interpreted as an attempt of the muscle fiber to maintain its functionality in a scenario of hypoxia, lipidosis and fibrosis (Petracci et al., 2019). The higher concentration of ribonuclease/angiogenin inhibitor 1 (RNH1) reveals that muscle fibers from WS chicken breasts were subjected to oxidative stress and struggled for survival. RNH1 controls the activity of angiogenin, which is known to play a role in cell growth under both, physiological and pathological conditions. The upregulation of RNH1 typically occurs under situations of hypoxic and oxidative stress that leads to the accumulation of oxidized and aggregated proteins (Pizzo et al., 2013). This interpretation is sound given the significantly higher concentration of allysine and Schiff bases in proteins from WS chicken breasts that in the normal counterparts. Furthermore, the overexpression of the Kelch-like protein family member 41 confirms the concurrence of oxidative stress, intracellular accumulation of oxidized proteins and impaired homeostasis. Kelch proteins are implicated in protein ubiquitination in the process of tagging damaged proteins to be eventually degraded in a coordinated turnover (Gupta & Beggs, 2014). The upregulation of this protein shows a cellular attempt to homeostasis maintenance by repairing oxidized proteins in a likely scenario of ROS overproduction.

4. Conclusions

Oxidative stress seems play an important role in chicken WS myopathy and involves thiols depletion, and malondialdehyde, allysine, Schiff base formation. The results presented here indicate that lipids and proteins from WS chicken breast meat are more susceptible to oxidative damage due to impaired of activity SOD, CAT and GPX endogenous antioxidant enzymes on the muscle tissue. The proteomic study reveals a failed attempt to maintain the biological function of the muscle fibre and the redox homeostasis. The accumulation of oxidized proteins may be a major pathway in the onset of the myopathy as proteins involved in cellular repair and protein turnover are upregulated.

Acknowledgements

The authors acknowledge the CNPq – Brazilian National Council for Scientific and Technological Development (Process number 430832/2016-8).

References

Adabi, S. G., & Soncu, E. D. (2019). White striping prevalence and its effect on meat quality of broiler breast fillets under commercial conditions. *Journal of Animal Physiology and Animal Nutrition*, 103(4):1060–1069.

Alfaia, C. M. M., Alves, S. P., Lopes, A. F., Fernandes, M. J. E., Costa, A. S. H., Fontes, C. M. G. A., Castro, M. L. F., Bessa, R. J. B., & Prates, J. A. M. (2010). Effect of cooking methods on fatty acids, conjugated isomers of linoleic acid and nutritional quality of beef intramuscular fat. *Meat Science*, 84:769–777.

AOAC (2000). *Official Methods of Analysis*. Gaithersburg, Maryland, USA: Association of Official Analytical Chemists. Methods 981.10, 950.46, 920.153, 990.26.

Brambila, G. S., Bowker, B. C., & Zhuang, H. (2016). Comparison of sensory texture attributes of broiler breast fillets with different degrees of white striping. *Poultry Science*, 95:2472–2476.

Brancaccio, P., Lippi, G., & Maffulli, N. (2010). Biochemical markers of muscular damage. *Clinical Chemistry and Laboratory Medicine*, 48(6): 757–767.

Carvalho, R. H., Ida, E. I., Madruga, M. S., Martínez, S. L., Shimokomaki, M., & Estévez, M. (2017). Underlying connections between the redox system imbalance, protein oxidation and impaired quality traits in pale, soft and exudative (PSE) poultry meat. *Food Chemistry*, 215:129–137.

Chelh, I., Gatellier, P., & Santé-Lhoutellier (2007). Characterisation of fluorescent Schiff bases formed during oxidation of pig myofibrils. *Meat Science*, 76:210–215.

Delgado, J., Núñez, F., Asensio, M. A., & Owens, R. A. (2019). Quantitative proteomic profiling of ochratoxin A repression in Penicillium nordicum by protective cultures. *International Journal of Food Microbiology,* 305:108243.

Doherty, M. K., McLean, L., Hayter, J. R., Pratt, J. M., Robertson, D. H. L., El-Shafei, A., Gaskell, S.J., & Beynon, R. J. (2004). The proteome of chicken skeletal muscle: Changes in soluble protein expression during growth in a layer strain. *Proteomics*, 4(7), 2082–2093.

Estévez, M. (2011). Protein carbonyls in meat systems: A review. *Meat Science*, 89(3), 259–279.

Estévez, M. (2015). Oxidative damage to poultry: from farm to fork. *Poultry Science*, 94(6), 1368–1378.

Estévez, M., & Xiong, Y. (2019). Intake of oxidized proteins and amino acids and causative oxidative stress and disease: recent scientific evidences and hypotheses. *Journal of Food Science*, 84(3):387–396.

Estévez, M., Geraert, P.-A., Liu, R., Delgado, J., Mercier, Y., & Zhang, W. (2020). Sulphur amino acids, muscle redox status and meat quality: More than building blocks – Invited review. *Meat Science*, 108087.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A Simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226(1):497–509.

Ganhão, R., Estévez, M., & Morcuende, D. (2011). Suitability of the TBA method for assessing lipid oxidation in a meat system with added phenolic-rich materials. *Food Chemistry*, 126(2), 772–778.

Garcia-Garcia, A., Rodriguez-Rocha, H., Madayiputhiya, N., Pappa, A., I. Panayiotidis, M., & Franco, R. (2012). Biomarkers of Protein Oxidation in Human Disease. *Current Molecular Medicine*, 12(6), 681–697.

Gupta, V.A., & Beggs, A.H. (2014). Kelch proteins: emerging roles in skeletal muscle development and diseases. *Skeletal Muscle*, 4:11.

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science*, 71:194–204.

Kosakowska, M., Pietraszek-Gremplewics, K., Jozkowicz, A., & Dulak, J. (2015). The role of oxidative stress in skeletal muscle injury and regeneration: focus on antioxidant enzymes. *Journal of Muscle Research and Cell Motility*, 36(6):377–393.

Kuttappan, V. A., Brewer, V. B., Apple, J. K., Waldroup, P. W., & Owens, C. M. (2012). Influence of growth rate on the occurrence of white striping in broiler breast fillets. *Poultry Science*, 91:2677–2685.

Kuttappan, V. A., Shivaprasad, H. L., Shaw, D. P., Valentine, B. A., Hagis, B. M., Clark, F. D., McKee, S. R., & Owens, C. M. (2013). Pathological changes associated with white striping in broiler breast muscle. *Poultry Science*, 92:331–338.

Kuttappan, V. A., Owens, C. M., Coon, C., Hargis, B. M., Vazquez-Añon, M. (2017). Incidence of broiler breast myopathies at 2 different ages and its impact on selected raw meat quality parameters. *Poultry Science*, 00:1–5.

Luber, C.A., Cox, J., Lauterbach, H., Fancke, B., Selbach, M., Tschopp, J., Akira, S., Wiegand, M., Hochrein, H., O'Keeffe, M., & Mann, M. (2010). Quantitative proteomics reveals subset-specific viral recognition in dendritic cells. *Immunity*, 32(2), 279–289.

Marklund, S., & Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, 47:469–474.

Meloche, K.J., Fancher, B.I., Emmerson, D.A., Bilgili, S.F., & Dozier, W.A. (2018). Effects of reduced dietary energy and amino acid density on Pectoralis major myopathies in broiler chickens at 36 and 49 days of age. *Poultry Science*, 97:1794–1807.

Mudalal, S., Babini, E., Cavani, C., & Petracci, M. (2014). Quantity and functionality of protein fractions in chicken breast fillets affected by white striping. *Poultry Science*, 93:1–9.

Mudalal, S., Lorenzi, M., Soglia, F., Cavani, C., & Petracci, M. (2015). Implications of White striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. *Animal*, 9(4):728–734.

Muller, F. L., Lustgarten, M. S., Jang, Y., Richardson, A., & Remmen, H. V. (2007). Trends in oxidative aging theories. *Free Radical Biology & Medicine*, 43:477–503.

OECD, (2020). Meat consumption (indicator). doi: 10.1787/fa290fd0-en (Accessed on 24 February 2020).

Petracci, M., Mudalal, S., Bonfiglio, A., & Cavani, C. (2013). Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. *Poultry Science*, 92:1670–1675.

Petracci, M., Mudalal, S., Babini, E., & Cavani. C. (2014). Effect of white striping on chemical composition and nutritional value of chicken breast meat. *Italian Journal of Animal Science*, 13:3138.

Petracci, M., Soglia, F., Madruga, M., Carvalho, L., Ida, E., & Estévez, M. (2019). Wooden-breast, white striping, and spaghetti meat: Causes, consequences and consumer perception of emerging broiler meat abnormalities. *Comprehensive Reviews in Food Science and Food Safety*, 18(2):565–583.

Pizzo, E., Sarcinelli, C., Sheng, J., Fusco, S., Formiggini, F., Netti, P., Yu, W., D'Alessio, G.,& Hu, G.-f. (2013). Ribonuclease/angiogenin inhibitor 1 regulates stress-induced subcellular localization of angiogenin to control growth and survival. *Journal of Cell Science*, 126(18), 4308–4319.

Rysman, T., Jongberg, S., Royen, G. V., Weyenberg, S. V., Smet, S. D., & Lund, M. N. (2014). Protein thiols undergo reversible and irreversible oxidation during chill storage of ground beef as detected by 4,4′-dithiodipyridine. *Journal of Agricultural and Food Chemistry*, 62:12008–12014.

Soglia, F., Laghi, L., Canonico, L., Cavani, C., & Petracci, M. (2016). Functional property issues in broiler breast meat related to emerging muscle abnormalities. *Food Research International*, 89:1071–1076.

USDA (2019). Poultry- Production and value, 2018 Summary. ISSN: 1949-1573.

Utrera, M., Morcuende, D., Rodríguez-Carpena, J-G., & Estévez, M. (2011). Fluorescent HPLC for the detection of specific protein oxidation carbonyls-σ-aminoadipic (AAS) and γ-glutamic (GGS) semialdehydes-in meat systems. *Meat Science*, 89(4):500–506.

Utrera, M., Rodríguez-Carpena, J. G., Morcuende, D., & Estévez, M. (2012). Formation of lysine-derived oxidation products and loss of tryptophan during processing of porcine patties with added avocado byproducts. *Journal of Agricultural and Food Chemistry*, 60:2917-39–26.

Zambonelli, P., Zappaterra, M., Soglia, F., Petracci, M., Sirri, F., Cavani, C., & Davoli, R. (2017). Detection of differentially expressed genes in broiler pectoralis major muscle affected by White Striping – Wooden Breast myopathies. *Poultry Science*, 95(12): 2771–2785.

Zhu, X., Ruusunen, M., Gusella, M., Zhou, G., & Puolanne, E. (2011). High post-mortem temperature combined with rapid glycolysis induces phosphorylase denaturation and produces pale and exudative characteristics in broiler pectoralis major muscles. *Meat Science*, 89:181–18.

FIGURE CAPTION

Figure 1. Proposal of involved mechanisms in the onset of the WS myopathy (Designed with information found in Petracci et al. (2019) along with results from the present study).

Table 1. Physical-chemical properties of normal chicken breast and those affected by the WS myopathy in the moderate and severe degrees.

	Normal	WS-M	WS-S	p
Moisture[1]	75.75±0.66	75.70±1.04	75.75±1.07	ns
Protein[1]	21.65a±0.69	20.65ab±0.99	20.28b±0.82	*
Lipid[1]	2.56b±0.54	4.03a±0.65	4.10a±0.80	***
Collagen[1]	0.38b±0.05	0.45a±0.04	0.49a±0.06	**
pH	5.60b±0.17	5.92a±0.17	5.88a±0.14	*
WHC[2]	29.42b±3.35	31.88ab±2.58	35.61a±3.13	*
L*	59.58a±1.91	56.22b±1.74	56.08b±3.33	*
a*	2.31b±0.33	3.12a±0.47	3.65a±0.51	*
b*	5.73b±1.51	7.27ab±2.12	9.75a±2.74	**
Hardness[3]	63.46c±8.98	98.10a±10.58	71.80b±7.51	*

[1] Results expressed as g/100 muscle

[2] Results expressed as percentage of retained water

[3] Results expressed as Newtons

[a,b] Significance in ANOVA: *p<0.05; **: p<0.01; ***: p<0.001; ns: no significant

Table 2. Markers of oxidative damage in normal chicken breast and those affected by the WS myopathy in the moderate and severe degrees.

	Normal	WS-M	WS-S	p[A]
TBARS[1]	0.22c±0.08	0.37b±0.09	0.64a±0.19	***
Free thiols[2]	2.32a±0.39	2.18a±0.31	0.68b±0.21	***
Allysine[3]	1.99b±0.39	2.29ab±0.47	3.15a±0.81	**
Schiff Bases[4]	258b±54	441a±65	645a±80	***
Disulphide bonds[2]	3.17b±0.51	5.41a±0.65	2.84c±0.41	*

[1] Results expressed as mg MDA/kg muscle

[2] Results expressed as μM

[3] Results expressed as nmol allysine/mg protein

[4] Results expressed as fluorescence intensity

[a,b] Significance in ANOVA: *p<0.05; **: p<0.01; ***: p<0.001

Table 3. Enzyme antioxidant activities in normal chicken breast and those affected by the WS myopathy in the moderate and severe degrees.

	Normal	WS-M	WS-S	p[A]
CAT[1]	40.1a±6.3	45.7a±4.8	23.6b±4.2	***
GPX[2]	0.54b±0.18	0.87a±0.21	0.21c±0.09	***
SOD[3]	73.1a±9.9	75.4a±7.5	56.2b±4.5	***

[1] Results expressed as μmol x min-1 x g-1 (U/g)

[2] Results expressed as μmol of oxidized NADPH μL -1 min g-1 (U/g)

[3] Results expressed as as U/g of sample, SOD activity that inhibits the reaction by 50%.

[a,b] Significance in ANOVA: ***: $p<0.001$

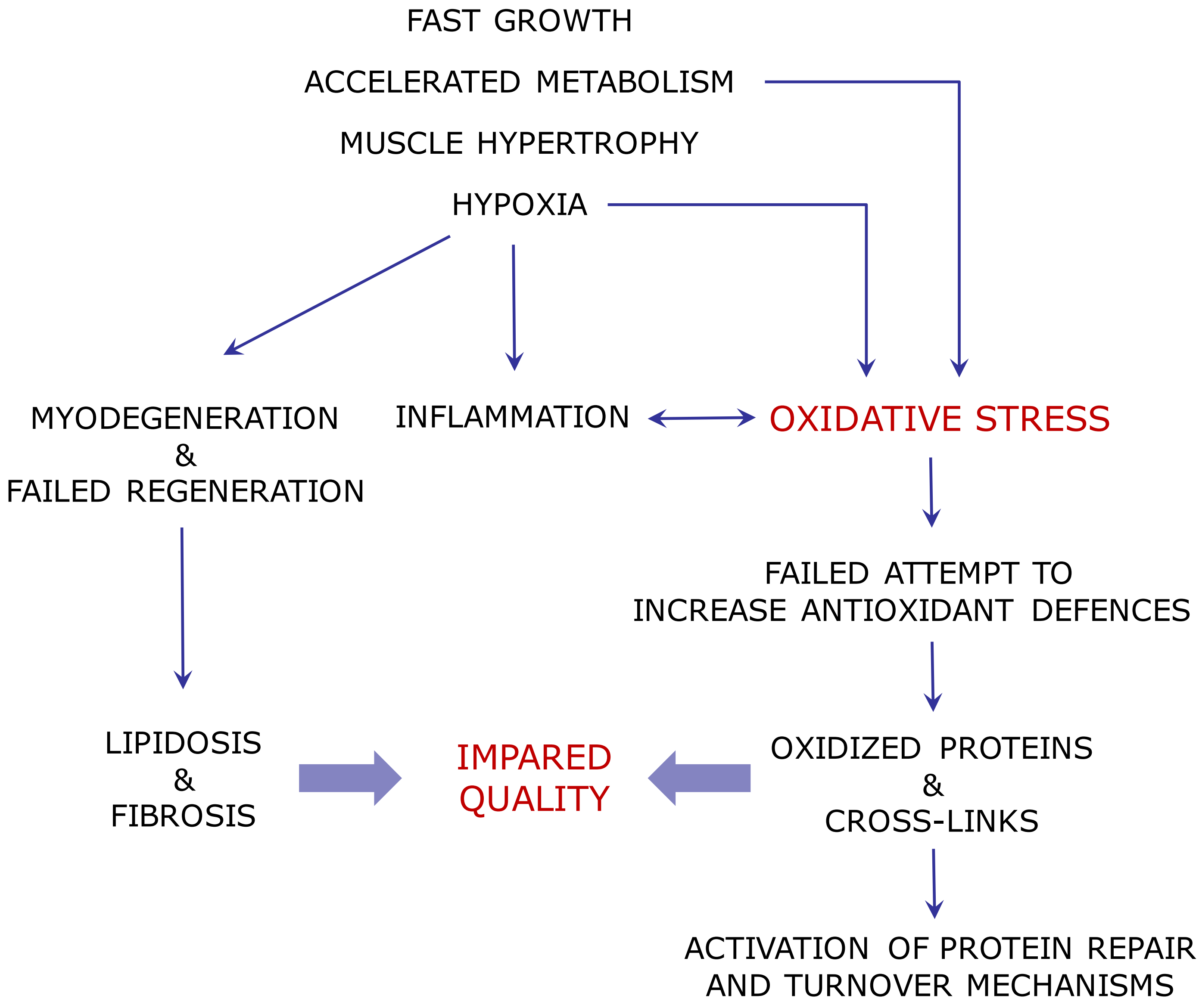
Table 4. Discriminating proteins between normal and WS-S chicken muscle breasts.

Protein	p-values	Fold-change[1]	Biological function	FASTA accession number
Voltage-dependent anion channel	-	S	ATP synthesis	A0A1L1RVM8
Collagen alpha-3(VI) chain	-	S	Cell adhesion	A0A1D5PGD5
14-3-3 protein zeta	-	S	Regulatory binding protein	A0A1L1RRT9
Desmoplakin	-	S	Cell adhesion	E1BWI0
Alpha-actinin-1	-	S	Actin cross-link formation	A0A1D5P9P3
Troponin T, fast skeletal muscle isoforms	-	S	Muscle contraction	P12620
Uncharacterized protein	-	S	Indefinite	F1NZJ2
Troponin C, skeletal muscle	0.010	5.49	Muscle contraction	P02588
Calsequestrin-2	0.015	3.90	Muscle contraction regulation	P19204
Troponin I, fast skeletal muscle	0.007	3.36	Muscle contraction	P68246
14-3-3 protein epsilon	0.042	2.74	Regulatory binding protein	Q5ZMT0
Troponin T variant TnTx7-e16	0.024	2.72	Muscle contraction regulation	O57559
Alpha-actinin-2	0.042	2.33	Actin cross-link formation	R9PXQ0
Ribonuclease/angiogenin inhibitor 1	0.006	2.29	Cell redox homeostasis	Q5ZIY8
Sarcalumenin	0.017	2.18	GTP binding	A0A1D5P984
Annexin	0.015	2.15	Calcium ion binding	A0A1D5PY67
Kelch like family member 41	0.030	2.00	Ubiquitination/Proteasome activation	A0A1D5P0B5
Creatine kinase S-type, mitochondrial	0.037	1.90	ATP binding	P11009
Uncharacterized protein	0.023	1.34	Indefinite	F1NZ04
Sarcoplasmic/endoplasmic reticulum calcium ATPase 1	0.018	1.31	Muscle contraction regulation	P13585

L-lactate dehydrogenase	0.018	0.82	Carbohydrate metabolic process	E1BTT8
Creatine kinase M-type	0.022	0.78	ATP synthesis	P00565
Pyruvate kinase PKM	0.002	0.77	Glycolytic process	P00548
Uncharacterized protein	0.038	0.46	Indefinite	E1C7I7
Uncharacterized protein	-	N	Indefinite	A0A1D5NUS2

[1] S: found only in WS-S chicken breast muscles. N: found only in normal chicken breast muscles.

677 **Figure 1.**


FAST GROWTH
ACCELERATED METABOLISM
MUSCLE HYPERTROPHY
HYPOXIA
MYODEGENERATION & FAILED REGENERATION
INFLAMMATION
OXIDATIVE STRESS
FAILED ATTEMPT TO INCREASE ANTIOXIDANT DEFENCES
LIPIDOSIS & FIBROSIS
IMPARED QUALITY
OXIDIZED PROTEINS & CROSS-LINKS
ACTIVATION OF PROTEIN REPAIR AND TURNOVER MECHANISMS

4. 3 ARTIGO III – CONSUMERS AWARENESS OF WHITE-STRIPING AS A CHICKEN BREAST MYOPATHY AFFECTS THEIR PURCHASING DECISION AND EMOTIONAL RESPONSES

O artigo foi submetido ao periódico Food Science and Technology - LWT em 12 de dezembro de 2019, sob o título *Consumers awareness of White-Striping as a chicken breast myopathy affects their purchasing decision and emotional responses* (ANEXO D).

Consumers Awareness of White-Striping as a Chicken Breast Myopathy Affects Their Purchasing Decision and Emotional Responses

L. Carvalho[1], S. Ventanas[2], L. Souza[1]; M. Madruga[1], M. Estévez[2, *]

[1] Postgraduate program in Food Science and Technology. Department of Food Engineering, Federal University of Paraiba, João Pessoa, Paraíba, Brazil

[2] Institute of Meat and Meat Products (IPROCAR), TECAL Research Group, University of Extremadura, Cáceres, Spain.

*Corresponding autor: Mario Estévez. Institute of Meat and Meat Products (IPROCAR), University of Extremadura, Avd. Universidad, sn. 10003, Cáceres, Spain. Phone number: +34 927251390. Email: mariovet@unex.es

Abstract

The aim of this study was to evaluate consumers' degree of knowledge, acceptability, and purchase intention of raw chicken breasts affected by the white striping (WS) myopathy (Moderate [WS-M] and Severe [WS-S]) as compared to Normal (N). The emotions evoked in consumers by roasted WS-S and N chicken breasts in two different consumption situations (before [BI] and after [AI] being informed of WS condition) was also assessed. WS samples presented lower acceptability and purchase intent compared to N, in both BI and AI situations. Providing information on the myopathy (AI) led to a significant reduction in acceptability of WS-M and WS-S samples, and a significant increase in consumers rejection of the same samples. Upon cooking, consumers detected no differences between WS-S and N chicken breasts for color, flavor and overall acceptability and the informed situation (BI vs AI) had a negligible impact. However, the WS-S samples had higher scores for odor and texture parameters compared to N. WS myopathy increases the emotions "interested" and "enthusiastic", although no major differences in the emotional profile were observed. The degree of knowledge about WS myopathy was inversely proportional to the consumer's acceptability and purchase intention.

Keywords: Chicken breast; white-striping; consumers; acceptability; emotions.

1. Introduction

Poultry production is the fastest growing meat sector, increasing 4.7 percent in 2010 to 98 million tons and poultry consumption has dramatically trended higher over the last 40 years in the US peaking around 115 lbs per capita in 2018 (USDA, 2018). Meeting the increasing global demand for chicken meat has been made by optimizing broiler production and selecting broilers for fast growth and high carcass yields (Petracci et al. 2015). Havenstein et al. (2003) already emphasized the extraordinary achievements made over the last 50 years in terms of feed conversion rates and growth rates in broilers. During this period, the body weight of modern Ross 308 broiler at 42 days of age is more than 4 times higher than that of an Athens-Canadian Randombred Control strain raised in the 50's. (~ 2.4 kg vs. 0.55 kg). As a likely result of pushing biological boundaries to the limit, spontaneous myopathies have arisen in broilers and turkeys (Petracci et al., 2019) Chicken breast abnormalities such as white-striping (WS), wooden breast (WB) and spaghetti meat (SM) are closely associated with intensive and exhausting animal production systems (Mutryn et al., 2015; Petracci et al., 2015).

The occurrence of white striations along the direction of the muscle fibers makes chicken breasts affected by WS easily recognizable. The white stripes are revealed at the microscopy as accumulation of lipids and connective tissue (Kuttappan et al., 2013a). Unlike the typical marbling fat (intramuscular fat), observed in certain pork and beef muscles, these striations do not respond to a physiological accretion of lipids that may improve meat quality. Conversely, it is regarded as a reflection of a myodegeneration process that, in addition to the increased deposition of fat and connective tissue, involves interstitial inflammation, edema, infiltration of inflammatory cells and necrosis (Kuttappan et al., 2013a,b). Among the aforementioned myopathies, WS is the most common with more than 98% of birds at 9 weeks of age assessed in the US, displaying symptoms of the myopathy. According to this study, more than 55% classified as moderate and severe cases (Kuttappan et al., 2017).

Since consumers are the eventual recipients of poultry meat products, their attitude towards these myopathies should be regarded as a relevant issue. According to several studies, WS condition may not imply safety concerns and only certain physico-chemical parameters are modified such as moisture, fat and collagen content (Baldi et al., 2019). The sensory properties of chicken breasts do not seem to be seriously affected by WS beyond the unusual occurrence of the white striations. Yet, it is largely ignored, the extent to which consumers of chicken meat are aware of the origin of those striations or if they even pay attention to this uncommon feature. To our knowledge, only one research work, limited to a hedonic study, has dealt with consumer preferences on chicken breast muscles affected by WS.

To gain further insight into the consumer's attitude towards emerging chicken breast myopathies, innovative sensory techniques may be applied. The study of the emotions elicited by foods has recently attracted considerable attention in sensory and consumer science. Food-evoked emotions enable a deeper understanding of food choice and consumer behavior (Jiang et al., 2014). This novel sensory tool has been scarcely applied to meat products and only few recent studies report the emotions evoked during consumption of meat products (Merlo et al., 2019). The appearance of the white striations could be enough motivation for the consumer to reject breast meat affected by WS. However, the awareness of the fundamental causes of WS and the recognition of the breast as a 'sick muscle' may affect the emotions evoked in the consumers, their attitude and eventual purchasing decision. This research study was designed to proven whether this hypothesis is correct or not.

2. Material and methods

2.1. Samples, identification and classification

For the present study, chicken breasts were purchased at four local supermarkets in Caceres, Spain. Samples were allocated to one of the following three groups based on the criteria described by Kuttappan et al. (2012a): Normal ([N] did not show white striation on breast surface), WS-moderate ([WS-M] exhibited white striations with < 1 mm thickness), and WS-severe ([WS-S] exhibited white striations with > 1 mm thickness). Sixty chicken breasts (n=20 of each type) were collected and used for the present study.

2.2. Physico-chemical composition

Protein, moisture, ash and collagen contents were analyzed in Normal, WS-moderate and WS-severe meats, according to the Association of Official Analytical Chemists (AOAC, 2000a, 2000b, 2000c, 2000d). The fat content was determined according to the methodology described by Folch et al. (1957).

2.3. Experimental design and sensory evaluations

2.3.1. Acceptability test and purchasing decision of raw chicken breasts

A total of 101 regular consumers and purchasers of chicken breast (64% female, 36% male, aged 18-61) took part in the study. They were recruited among staff, students and visitors at the University Campus at University of Extremadura (Caceres, Spain). This study consisted of two experiments, each of which used a different approach to measure acceptability and purchase intent based on visual appearance of the raw samples (N, WS-M and WS-S).

In the first experiment (non-informed scenario), consumers performed a blind evaluation of the samples (no information about the myopathy was provided, BI). The samples were presented coded with 3-digit numbers without any type of additional information.

In the second experiment (informed scenario), the same consumers performed an informed evaluation of the samples (AI). The participants evaluated similar samples to the first experiment, coded with 3-digit numbers, but in this occasion, samples were identified as N, WS-M and WS-S and general information on the white-striping condition was provided (available as Supplementary material).

In both (BI and AI) experiments, the samples were presented to participants simulating retail conditions in supermarket. The acceptability and purchase intention were evaluated by consumers based on visual appearance of the raw breast poultry meat using a non-structured five-point hedonic scale, with 1 being ‘disliked extremely’ or ‘definitely would not buy it’, and 5 being ‘liked extremely’ or ‘definitely would buy it’.

2.3.2. Acceptability and emotions evoked from roasted chicken breasts

This study was carried out with 46 regular Spanish consumers of poultry meat (aged: 20 to 61). They were recruited among staff, students and visitors at the University Campus at University of Extremadura (Caceres, Spain) who did not participate in the aforementioned assessment of the raw samples. In this experiment, chicken breast samples (N and WS-S) were roasted in oven at 180 °C until reaching internal temperature of 75ºC. Subsequently, the breasts were sliced (1.5 cm of thickness) and submitted for sensorial analysis under two experimental conditions.

In the first experiment (non-informed scenario), consumers were asked to state the acceptability of particular eating quality traits and the emotional responses evoked upon consumption of roasted chicken samples under a blind condition (before information – BI). The samples were presented coded with 3-digit numbers and no additional information was provided.

In experiment 2, the same approach was performed; however, samples were presented to consumers identified as N or WS-S together with a short report on the WS myopathy (after information – AI). In this particular case, given the samples to be assessed were already roasted, the report included pictures of the fresh chicken breasts (report available as Supplementary material).

In both BI and AI experiments, consumers were asked to evaluate the acceptability of the samples based on their appearance (color), odor, flavor, texture, and overall acceptability using a 5-point scale from 1= disliked extremely to 5= liked extremely. The emotional responses were assessed by using a rate-all-that-apply (RATA) questionnaire based on a list of 25 emotional terms (active, adventurous, aggressive, bored, calm, disgusted, enthusiastic, free, joyful, good, good-natured, guilty, happy, interested, loving, nostalgic, pleasant, satisfied, secure, tame, tender, understanding, warm, wild, worried; EsSence25 methodology) developed by Nestrud et al. (2016) and translated into Spanish by Dorado et al. (2016). Consumers identified and rated the intensity of the emotions using a 5 point intensity scale anchored from 'not at all' to 'extremely'.

2.4. Statistical analyses

In this study, a Shapiro-Wilk normality test ($\alpha = 0.05$) was performed. ANOVA was applied to the normal data, and the means were compared to Tukey test ($p < 0.05$). The Kruskal-Wallis nonparametric test ($p < 0.05$) was applied to non-normal data for comparing three or more samples, and the means were compared through the Dunn's test at an overall alpha level of 0.05, and Mann-Whitney U nonparametric test ($p < 0.05$) was applied to non-normal data for comparing two samples. The results of acceptability and purchase intent tests, based on the visual appearance of raw meat, were expressed in a histogram of the responses, indicating the percentage of assessors that marked the alternatives presented. Principal component analysis (PCA) was used for obtaining a bi-dimensional representation of samples and sensory acceptance was based on the consumption of the roasted meat. The responses obtained from RATA (Rate-All-That-Apply) questionnaire were evaluated by Cochran's Q test ($p < 0.05$). A bi-dimensional representation of samples and emotional responses was obtained using correspondence analysis (CA). Statistical data processing and graphic construction were performed with the XLStat software version 2014.5.03 (Addinsoft, New York, USA) for Microsoft Excel, and GraphPad Prism software version 6.01 (Graphpad Software Inc., San Diego, California, USA).

3. Results and Discussion

3.1 Physico-chemical composition

The results for proximate composition are shown in Table 1. There were no differences among the groups for the contents of moisture and ash. The myopathy condition, however, had a significant effect on the fat, protein and collagen contents. The N chicken breast exhibited significantly higher ($p = 0.0044$) protein values compared to breasts affected by the WS myopathy. WS-M and WS-S exhibited greater ($p < 0.0001$) lipid content than the N breast. The lipid content varied from 2.56 g/100g (N) to 4.10 g/100g (WS-S). The highest and the lowest content of collagen were found in WS (moderate: 0.46 g/100g; severe: 0.49 g/100g) and in N breasts (0.39 g/100g), respectively.

The decrease in the protein content and the consistent increase in fat content in WS meats, may be explained by the degeneration or atrophy of muscle fiber, resulting in accumulation of fat (lipidosis) (Petracci et al., 2019). The higher collagen content in the samples affected by the myopathy can be explained by the reparative fibrotic responses (fibrosis) (Kuttappan et al., 2012b; Mudalal et al., 2014; Petracci et al., 2014).

3.2. Consumer profile and level of myopathy recognition

In order to carry out the sensory assessment of acceptability and purchase decision (appearance of raw meat), a group of 101 consumers were recruited. Among these, 98.0% were usual consumers of chicken breast and 92.9% were usual purchasers of this type of meat. The age of the evaluators ranged as follows: 18–23 years old (67.3%); 24–31 years old (13.9%); 32–41 years old (8.9%); 42–51 years old (4,0%); 52 to 61 years (5,9%). A relatively low proportion of consumers (16%), was aware of the WS condition, while the remaining 84 % stated to have never heard of it. The information available on the literature on the level of awareness on this myopathy among usual consumers of chicken meat is very poor. This study proves that a large proportion of consumers ignore the incidence of this problem and the causes and consequences of the occurrence of white striations on the surface of chicken breasts. Interestingly, a low percentage of consumers belonging to the group unaware of the WS myopathy (2.4%) preferred chicken breasts affected by this pathological condition. Visually, they considered that the appearance of WS breasts was similar to marbled pork, assuming that visual lipids may be responsible for a higher meat quality. After raising awareness of the myopathy in the informed (AI) situation, 4.95% of the aforementioned consumers decided to reject WS samples for understanding that such condition may be linked to "*health problems*" with "*animal stress*" and "*suffering*". Further information on the impact of the AI situation on consumers' acceptability, purchasing decision and emotional responses will be provided in the following sections.

All the 46 consumers, who participated in the acceptability of the roasted samples and the assessment of emotion responses, declared to be usual consumers of chicken meat and 97.8% recognized themselves and usual purchasers of this type of meat. The age of the consumers ranged as follows: 18–23 years old (23.9%); 24–31 years old (32.6%); 32–41 years old (21.7%); 42–51 years old (8.7%); 52 to 61 years old (8.7%), while 4.4% did not report their age. All consumers accepted to assess the samples and hence, answered the questionnaire that evaluated of acceptability and emotional responses of cooked chicken samples under blinded evaluation conditions (BI). However, under the informed (AI) situation, 4.3% decided not to perform the test (Normal and WS-S), and 15.2% decided to perform the analysis of the N breasts only. These results illustrate the impact of the awareness of the myopathy condition on the consumers' attitude towards chicken meat consumption. Further detailed will be provided in the following sections.

3.3. Visual acceptability and purchase intention of raw chicken breast

The results from acceptability test based on visual appearance of raw meat are shown in Table 2. The WS samples presented lower acceptability compared to the N counterparts, in both non-informed (BI) and informed (AI) conditions ($p < 0.0001$). There were no differences between degrees of severity as data for WS-M and WS-S were similar. In addition, the results show that providing information on the WS condition to consumers (AI) caused a decrease in the acceptability as compared to the blind evaluation (BI). As expected, the different experimental conditions (BI vs. AI) did not affect the acceptance of N chicken breasts ($p = 0.5497$). Table 2 also shows the mean scores of purchase intent (PI) given by the consumers to the three types of raw chicken breasts (N, WS-M and WS-S), in the two evaluation conditions (BI vs. AI). Under both experimental conditions, WS breasts received lower PI compared to the N counterparts ($p < 0.001$). In accordance with the visual acceptability, no significant differences were observed between severity degrees of the myopathy. Likewise, the level of information affected the PI of chicken breasts affected by the WS myopathy. Arising awareness among consumers significantly decreased the PI scores in both the WS-M and WS-S (2.57 vs 2.29, and 2.72 vs 2.10, respectively). No significant differences were found for N chicken breast under different information scenarios ($p = 0.5564$). The consistency between visual acceptability and PI was reasonable given that the negative perception of consumers towards WS samples may affect their willingness to buy these products. A similar observation was made by Kuttappan et al. (2012c).

Fig. 1 shows the frequency histograms of the hedonic scale showing percentage of consumers providing a particular score to each type of chicken breast in both, BI and AI, situations. The percentage of consumers who “liked” N chicken breast increased from 75% to 82% in the informed situation, while the percentage of those who “disliked” N chicken decreased from 12% to 7% (Figure 1 - 1A vs. 1B). Yet, the most remarkable and significant effects of the informed situation (vs. non-informed) was observed for the chicken samples affected by the WS condition. Consistently with the mean scores of acceptability previously described, the percentage of consumers who “liked” WS-M (21%) and WS-S (31%) in the non-informed situation significantly decreased upon providing the information on the WS myopathy (10% and 7%, respectively) (Figure 1 - 2A vs. 2B and 3A vs 3C). Simultaneously, awareness of the WS condition increased the percentage of consumers who “disliked” WS-M (from 43% to 56%) and WS-S (from 35% to 70%). In the BI situation, the white striation in the WS samples was described by many consumers as “*non-normal appearance*” or “*fatty breast meat*”, providing these arguments as reasons for explaining their rejection in the BI situation. This rejection was aggravated after access to information as consumers. Consistent results were obtained for the frequency distribution of PI for poultry breasts under the BI and AI conditions (Figure 2). The percentage of consumers willing to buy N breast samples remained unchanged regardless of the information conditions (Figure 2 - 1A vs. 1B). However, the informed evaluation of samples resulted in a significant increase in "would not buy it" score, and a simultaneous reduction in the consumers PI for WS-M and WS-S meats compared to the results obtained in the blind condition. The percentage of consumers showing reluctance to buy WS-M and WS-S increased from 50% to 64% (Figure 2 - 2A vs. 2B), and from 45% to 72% (Figure 2 - 3A vs.3B).

The effect of the informed situation on the acceptability and PI of WS can be explained by several reasons. On the one hand, it may be attributed to moral cause. Since the occurrence of this myopathy is related to genetic selection of fast-growth breeds and the skeletal muscle eventually enters into a pathological situation, consumers alleged that such meat would come from animals which probably had “*health problems*” and suffered “*stress*” and “*compromised* *welfare*". In their recent study, Erian & Phillips (2017) report that most chicken consumers have a negative perception of intensive farming and animal welfare largely influences on their attitude towards chicken meat. On the other hand, the recognition of WS as a muscle pathology, may have negatively influenced the consumers behavior by associating the white stripes with unhealthy or unsafe meat, even though such association was ruled out in the report provided to consumers in the PI situation. Besides that, consumers may have associated the high fat content as nutritionally unhealthy, as concluded by Kuttappan et al. (2012c) in their study. According to Ogunwole and Adedeji (2014), consumers prefer lean meat to meats with moderate fat due to concerns about fat consumption and risk of cardiovascular diseases. These arguments are very much in line with current consumers' trends in reducing meat consumption, motivated by health, animal welfare or environmental concerns (Sanchez-Sabate & Sabaté, 2019).

3.4. Acceptability of roasted chicken breast

There were no differences between N and WS cooked meats for the color, flavor and overall acceptability under both, BI and AI, conditions (Table 3). Both types of meat received mean scores around 3 ('neither liked nor disliked') for all attributes evaluated, with these results indicating that consumers were indifferent to chicken samples. This attitude may be explained by the lack of salt or spices that may have affected the hedonic scores delivered by consumers. N breasts had lower odor scores compared to the WS-S counterparts under the BI situation (2.83 vs 3.17, $p = 0.0224$), while no differences were found when samples were assessed in the informed situation (2.86 vs 2.89, $p > 0.05$). These results are explained by a significant decrease of odor scores in the WS-S in the informed evaluation ($p = 0.0478$). Whereas the highest odor level in WS-S meat may be related to the higher lipid content and its connection with flavor formation (Kosawska et al., 2017), the influence of the informed situation likely led consumers to a biased assessment of the WS samples manifested in lowered odor scores. This finding was not observed for the assessment of texture as in both BI and AI, WS-S breasts received higher texture scores than N breasts, with these results being statistically significant in the informed situation (p = 0.0399). The preference of consumers towards the texture of WS-S samples was unexpected given the background in the scientific literature. Petracci et al. (2013) found lower shear force values in raw WS-S breasts than in the normal counterparts. Consistently, Brambila et al. (2016) reported that trained panelists scored chicken breasts affected by the WS condition with higher hardness values than normal chicken breasts. The toughness of WS chicken breasts is typically explained by the accretion of connective tissue in these samples (Petracci et al., 2014); with this result also found in the present study (Table 1). It is generally assumed that a tougher chicken breast would be rejected by consumers but the present results indicate otherwise. It may also be possible that the accumulation of lipids could have influenced juiciness in chicken breasts affected by this myopathy and that, in turn, improved the consumer acceptability of the texture properties.

The two-dimensional map shown in Figure 3 allowed the identification of acceptability trends. The explained variance by the first two components shows a remarkably high value (96.48%), ensuring the quality projection of results. The variability explained by component 1 between samples was mainly due to “overall acceptability”, “flavor”, and in lower proportion, to “texture”. The variability explained by component 2 came mainly from “color” and “odor”. N chicken breasts, in both experimental conditions, were grouped and opposed to most of the computed factors. Indeed, N (BI and AI) received the lowest scores for most attributes. On the opposite, WS-S breasts, in the non-informed situation, was isolated and close to “texture” factors. Finally, WS-S in the informed situation was isolated too, and close to “flavor” attributes. The tendency of roasted WS breasts to have higher scores on acceptability attributes may be related to the higher fat content, once lipids contribute for the flavor, aroma, tenderness and juiciness of meats. This analysis also confirms the influence of the awareness of the WS condition on the overall acceptability as the computed quality attributes allowed the discrimination between the WS-S samples before and after the providing consumers with information on this myopathy.

3.5. Emotions evoked by roasted chicken breasts

Table 4 shows the results of the Cochran's Q test, in which the citation frequencies of particular emotions evoked during consumption of N and WS-S chicken breast in two consumption situations (BI and AI) were compared. In the non-informed situation (BI), no significant differences were found between N and WS-SE samples which, evoked, in general, positive emotions such as "*enthusiastic*", "*happy*", "*pleasant*", "*good*", and "*satisfied*" (scores > 0.5). The negative emotions with a high citation frequency (score > 0.5) in this scenario were "*tame*", "*bored*" and "*disgusted*". After being informed of the myopathy (AI), consumers displayed a different emotional profile characterized by feeling less "*good-natured*", "*loving*", "*warm*", and "*nostalgic*". Unlike arising negative emotions such as "*worried*" or "*disgusted*", being aware of the myopathy led consumers to feel less "*tame*" and "*bored*" which emphasizes the interest of the consumer for this odd chicken breast. It is worth recalling that almost 1 out of 5 consumers who participated in the BI situation, decided not to eat the WS-S samples in the AI situation, alleging feeling "*disgust*" at the breasts affected by the myopathy. It is, hence, crucial to underline that emotional profiles shown in Table 4 belong to consumers who *dared* to eat the samples regardless of the information provided on the WS myopathy. The emotional profile of consumers of roasted N chicken breasts remained unchanged in both consumption scenarios.

No significant differences in emotions intensity were observed by RATA analysis. The emotional profile evoked by the two types of samples (N and WS-S) under the two scenarios (BI and AI) are shown in Figure 4. While no significant differences were detected, clear trends for some particular emotions were observed. In the non-informed scenario, WS-S tended to evoke positive emotions such as “*pleasant*”, “*good*” and “*adventurous*” more intensively which may be linked to the higher sensory scores given in the acceptability test of the WS-S roasted samples. Upon being aware of the WS condition, the intensity of such positive emotions clearly diminished as well as others such as “*secure*”, “*calm*” and “*satisfied*”.

4. Conclusions

Low is the extent to which Spanish consumers recognize the occurrence of white striping on the surface of chicken breast as an abnormality linked to an impaired growth of the muscle tissue. Even in this unawareness situation, consumers identify these white marks as uncommon for a chicken breast and that reduces the visual acceptability and purchase intent of WS chicken breasts. Providing information on the occurrence, causes and consequences of WS myopathy penalizes the level of acceptability and purchase intent of chicken breasts affected by the myopathy. The cooking process of sliced chicken breasts blurs the explicit visualization of the white stripes and upon consumption in the non-informed scenario, consumers tended to like more, the WS-S breasts than the N counterparts. Yet, upon receiving the information on the myopathy (informed scenario), 1 out of 5 consumers decided not to eat the samples again showing a straightaway rejection due to “disgust”. The emotional profile was significantly affected by the awareness of the myopathy as consumers felt less “*good-natured*”, “*loving*”, and “*warm*”, among others. Consumers’ prerogative to be provided with information on what they eat may compromise the acceptability, purchase intent and emotions evoked during consumption of chicken breasts affected by the WS myopathy.

Acknowledgements

The authors acknowledge the CNPq – Brazilian National Council for Scientific and Technological Development (Process number 430832/2016-8). Authors thanks the

Government of Extremadura (*Consejería de Economía e Infraestructuras*) and the European Union for the research project IB16043- Fondo Europeo de Desarrollo Regional, “*Una manera de hacer Europa*”.

References

AOAC. 2000a. Crude protein in meat. 981.10. In W. Horwitz (Ed.), Official method of analysis (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemists.

AOAC. 2000b. Determination of moisture content. 950.46. In W. Horwitz (Ed.), Official method of analysis (17th ed). Gaithersburg, Maryland: Association of Official Analytical Chemists.

AOAC. 2000c. Ash of meat. 920.153. In W. Horwitz (Ed.), Official method of analysis (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemists.

AOAC. 2000d. Hydroxyproline in meat and meat products. 990.26. In W. Horwitz (Ed.), Official method of analysis (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemists.

Baldi, G., Soglia, F., Laghi, L., Tappi, S., Rocculi, P., Tavaniello, S., Mucci, R., Maiorano, G., & Petracci, M. (2019). Comparison of quality traits among chicken breast eat affected by current muscle abnormalities. *Food Research International*, 115, 369-376.

Brambila, G. S., Bowker, B. C., & Zhuang, H. (2016). Comparison of sensory texture attributes of broiler breast fillets with different degrees of white striping. *Poultry Science*, 95k, 2472-2476.

Dorado, R., Pérez-Hugalde, C., Picard, A., & Chaya, C. (2016) Influence of first position effect on emotional response. *Food Quality and Preference*, 49, 189-196.

Erian, I. & Phillips, C.J.C. (2017). Public understanding and attitudes towards meat chicken production and relations to consumption. *Animals*, 7(3), 20.

Folch, J., Lees, M., & Stanley, G. H. S. (1957) A Simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, 226(1), 497-509.

Havenstein, G.B., Ferket, P.R. & Qureshi, M.A. (2003). Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. *Poultry Science*, 82, 1500-1508.

Jiang, Y., King, J.M. & Prinyawiwatkul, W. (2014). A review of measurement and relationships between food, eating behavior and emotion. *Trends in Food Science and Technology*, 36(1), 15-28

Kosowska, M., Majcher, A. M., & Fortuna, T. (2017). Volatile compouds in meat and meat products. *Food Science and Technology*, 37(1), 1-7.

Kuttappan, V. A., Lee, Y. S., Erf, G. F., Meullenet, J. F. C., McKee, S. R., & Owens, C. M. (2012a) Consumer acceptance of visual appearance of broiler breast meat with varying degrees of white striping. *Poultry Science*, 91, 1240-1247.

Kuttappan, V. A., Brewer, V. B., Apple, J. K., Waldroup, P. W., & Owens, C. M. (2012b) Influence of growth rate on the occurrence of white striping in broiler breast fillets. *Poultry Science*, 91, 2677-2685.

Kuttappan, V. A., Goodgame, S. D., Bradley, C. D., Mauromoustakos, A., Hargis, B. M., Waldroup, P. W., & Owens, C. M. (2012c). Effect of different levels of dietary vitamin E (DL-α-tocopherol acetate) on the occurrence of various degrees of white striping on broiler breast fillets. *Poultry Science*, 91, 3230-3235.

Kuttappan, V. A., Brewer, V. B., Mauromoustakos, A., McKee, S. R., Emmert, J. L., Meullenet, J. F., & Owens, C. M. (2013a) Estimation of factors associated with the occurrence of white striping in broiler breast fillets. *Poultry Science*, 92, 811-819.

Kuttappan, V. A., Shivaprasad, H. L., Shaw, D. P., Valentine, B. A., Hargis, B.M., Clark, F. D., Mckee, S. R, & Owens, C. M. (2013b) Pathological changes associated with white striping in broiler breast muscles. *Poultry Science*, 92, 331-338.

Kuttappan, V. A., Owens, C. M., Coon, C., Hargis, B. M., & Vazquez-Añon, M. (2017). Incidence of broiler breast myopathies at 2 different ages its impact on selected raw meat quality parameters. *Poultry Science*, 96(8), 3005-3009.

Lorido, L., Pizarro, E., Estévez, M., Ventanas, S. (2019). Emotional responses to the consumption of dry-cured hams by Spanish consumers: A temporal approach. *Meat Science*, 149, 126-133.

Merlo, T.C., Soletti, I., Saldaña, E., (...), Dargelio, M.D.B., Contreras-Castillo, C.J. (2019). Measuring dynamics of emotions evoked by the packaging colour of hamburgers using Temporal Dominance of Emotions (TDE). *Food Research International*, 124, 147-155.

Mudalal, S., Babini, E., Cavani, C., & Petracci, M. (2014). Quantity and functionality of protein fractions in chicken breast fillets affected by white striping. *Poultry Science*, 93, 1-9.

Mutryn, M. F., Brannick, E. M., Fu, W., Lee, W. R., & Abasht, B. (2015). Characterization of a novel chicken muscle disorder through differential gene expression and pathway analysis using RNA-sequencing. *Genomics*, 16, 399-417.

Nestrud, M. A., Meiselman, H. L., King, S. C., Lesher, L. L., & Cardello, A. V. (2016). Development of EsSense25, a shorter version of the EsSense Profile®. *Food Quality and Preference*, 48, 107–117.

Ogunwole, O. A., & Adedeji, B. S. (2014). Consumers' preference and perception of the different types of meat among staff and students of the University of Ibadan, Nigeria. *Journal of Agriculture and Environmental Sciences*, 3(2), 77-95.

Petracci, M., Mudalal, S., Bonfiglio, A., & Cavani, C. (2013) Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. *Poultry Science*, 92, 1670-1675.

Petracci, M., Mudalal, S., Babini, E., & Cavani, C. (2014). Effect of white striping on chemical composition and nutritional value of chicken breast meat. *Italian Journal of Animal Science*, 13, 179-183.

Petracci, M., Mudalal, S., Soglia, F., & Cavani, C. (2015). Meat quality fast-growing broiler chickens. *World's Poultry Science*, 71, 363-373.

Petracci, M., Soglia, F., Madruga, M., Carvalho, L., Ida, E., & Estévez, M. (2019) Wooden-breast, White Striping, and Spaghetti Meat: causes, consequences and consumer perception of emerging broiler meat abnormalities. *Comprehensive Reviews in Food Science and Food Safety*, 19, 565-583.

Sanchez-Sabate, R., & Sabaté, J. (2019). Consumer attitudes towards environmental concerns of meat consumption: a systematic review. *International Journal of Environmental Research and Public Health*, 16(7), 1-37.

USDA (2018). United States Department of Agriculture. Poultry - Production and Value 2018 Summary. Retreived from (10/15/2019) nass.usda.gov/Publications/Todays_Reports/reports/plva0519.pdf

Table 1. Physicochemical composition (means ± standard deviations) of Normal and WS poultry breasts.

Parameter	Poultry breast meat			*P*-value
	N	WS-M	WS-S	
Moisture g/100g	75.75±0.66	75.70±1.04	75.75±1.07	0.9888
Protein g/100g	21.65±0.70^{a}	20.64±1.00^{b}	20.28±0.83^{b}	0.0044
Lipid g/100g	2.56±0.54^{b}	4.03±0.66^{a}	4.10±0.80^{a}	< 0.001
Ash g/100g	1.06±0.09	1.01±0.11	1.10±0.10	0.1751
Collagen g/100g	0.39±0.02^{b}	0.46±0.05^{a}	0.49±0.06^{a}	0.0024

^{a,b} Mean values within the same parameter followed by different superscript letters significantly differ by the Tukey test ($P < 0.05$).

Table 2. Acceptability and purchase intent under blind (BI) or informed (AI) conditions based on visual appearance of raw Normal and WS poultry breasts.

Experimental conditions	Poultry breast meat			*P*-value
	N	WS-M	WS-S	
Acceptability				
BI	3.83±0.95 ^{aA}	2.74±0.87 ^{bA}	2.99±0.99 ^{bA}	< 0.0001
AI	3.94±0.80^{aA}	2.39±0.86^{bB}	2.16±0.90^{bB}	< 0.0001
P-value	0.5497	0.0067	< 0.0001	-
Purchase Intent				
BI	4.02±0.94 ^{aA}	2.57±1.05 ^{bA}	2.72±1.16 ^{bA}	< 0.0001
AI	4.06±1.01 ^{aA}	2.29±1.02 ^{bB}	2.10±1.16 ^{bB}	< 0.0001
P-value	0.5564	0.0465	< 0.0001	-

^{a,b} Means with different low case superscript in the same row differ significant by the Dunn,s test (P < 0.05).

^{A,B} Means with different superscript (capital letters) in the same column differ significant by the U de Mann-Whitney test ($P < 0.05$).

Table 3. Acceptability under blind (BI) or informed (AI) conditions of roasted Normal and WS chicken breasts.

Experimental conditions	Poultry breast meat		*P*-value
	N	WS-S	
Color			
BI	2.80±0.83	2.85±0.73	0.9863
AI	2.83±0.61	2.89±0.60	0.4869
P-value	0.8401	0.6935	-
Odor			
BI	2.83±0.77 ^{b}	3.17±0.77 ^{aA}	0.0224
AI	2.87±0.65	2.91±0.55 ^{B}	0.5549
P-value	0.7762	0.0478	-
Texture			
BI	2.78±1.05	3.09±0.94	0.1646
AI	2.63±0.90 ^{b}	2.93±0.77 ^{a}	0.0399
P-value	0.3658	0.3583	-
Flavor			
BI	2.76±0.92	2.96±0.94	0.4032
AI	2.83±0.88	2.98±0.74	0.3769
P-value	0.7452	0.7907	-
Overall acceptability			
BI	2.78±0.84	3.02±0.83	0.2554
AI	2.76±0.74	3.00±0.67	0.0832
P-value	0.7975	0.9728	-

^{a,b} Means with different superscript small letter in the same row differ significant by the U de Mann-Whitney test (P < 0.05).

^{A,B} Mean with different superscript capital letters in the same column differ significant by the U de Mann-Whitney test (P < 0.05).

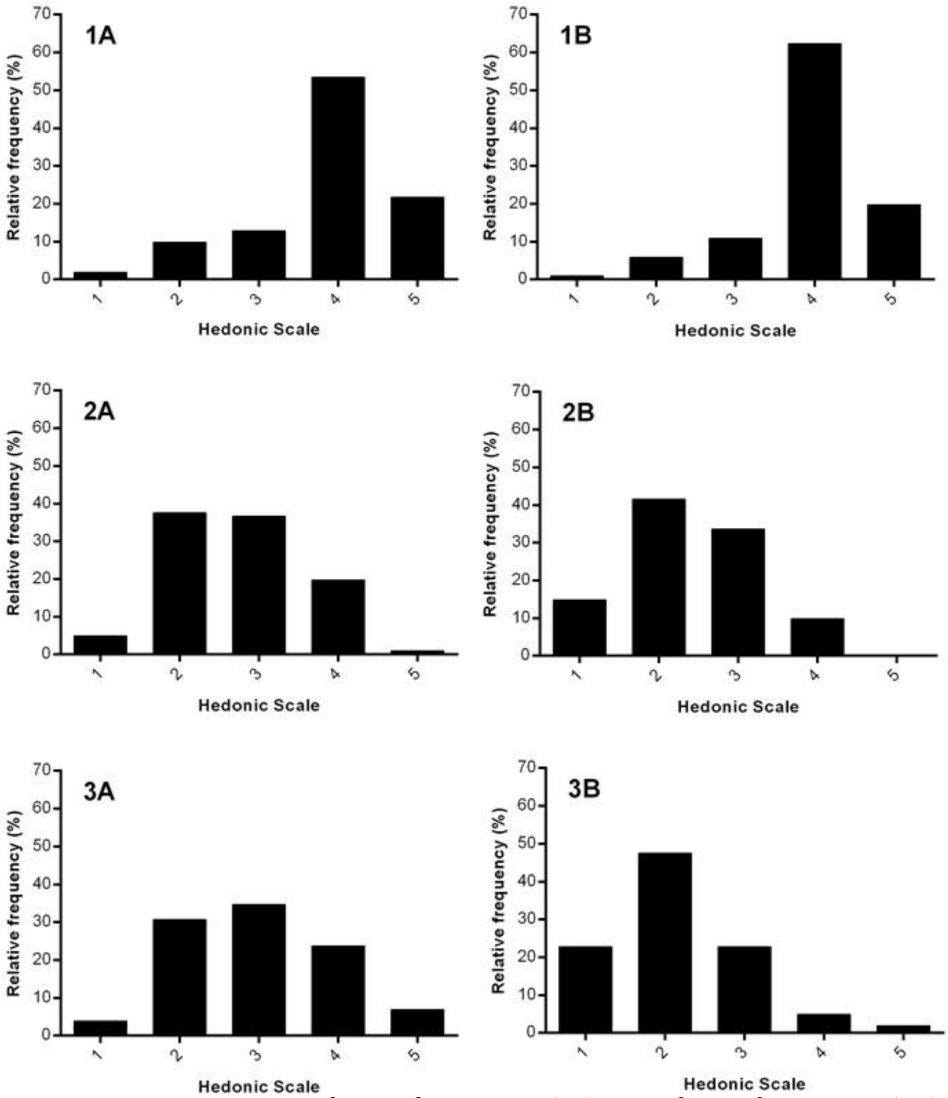
Table 4. Emotional Citation frequency of emotional terms evoked during consumption of roasted N and WS-S chicken breast, under blind (BI) and informed (AI) conditions.

Attributes	BI		AI		*P*-value
	Normal	WS-Sev	Normal	WS-Sev	
Enthusiastic	0.413 ^{ab}	0.478 ^{a}	0.304 ^{b}	0.261 ^{b}	0.0011
Happy	0.522	0.478	0.413	0.348	0.0865
Good-natured	0.370 ^{a}	0.348 ^{a}	0.304 ^{ab}	0.217 ^{b}	0.0110
Free	0.370 ^{a}	0.326 ^{ab}	0.304 ^{ab}	0.239 ^{b}	0.0513
Joyful	0.413	0.457	0.413	0.304	0.0775
Interested	0.478	0.478	0.500	0.435	0.8310
Understanding	0.413 ^{a}	0.370 ^{ab}	0.304 ^{ab}	0.261 ^{b}	0.0124
Pleasant	0.609	0.478	0.500	0.500	0.3360
Good	0.630	0.652	0.543	0.543	0.3449
Adventurous	0.326	0.370	0.283	0.261	0.0527
Secure	0.370	0.413	0.391	0.348	0.7141
Active	0.348	0.348	0.370	0.304	0.6729
Satisfied	0.543	0.630	0.543	0.478	0.2542
Loving	0.304 ^{ab}	0.326 ^{a}	0.283 ^{ab}	0.217 ^{b}	0.0148
Warm	0.304 ^{ab}	0.326 ^{a}	0.261 ^{ab}	0.217 ^{b}	0.0254
Calm	0.457	0.435	0.457	0.391	0.7090
Aggressive	0.326	0.348	0.304	0.239	0.0542
Nostalgic	0.348 ^{ab}	0.370 ^{a}	0.283 ^{ab}	0.239 ^{b}	0.0078
Wild	0.304 ^{ab}	0.326 ^{a}	0.283 ^{ab}	0.217 ^{b}	0.0148
Tame	0.565 ^{ab}	0.609 ^{a}	0.587 ^{a}	0.413 ^{b}	0.0111
Tender	0.348 ^{a}	0.326 ^{ab}	0.283 ^{ab}	0.217 ^{b}	0.0095
Guilty	0.304	0.326	0.304	0.239	0.1447
Worried	0.348	0.391	0.326	0.283	0.3149
Bored	0.609 ^{a}	0.609 ^{a}	0.543 ^{ab}	0.370 ^{b}	0.0006

Disgusted	0.522	0.500	0.609	0.435	0.1274

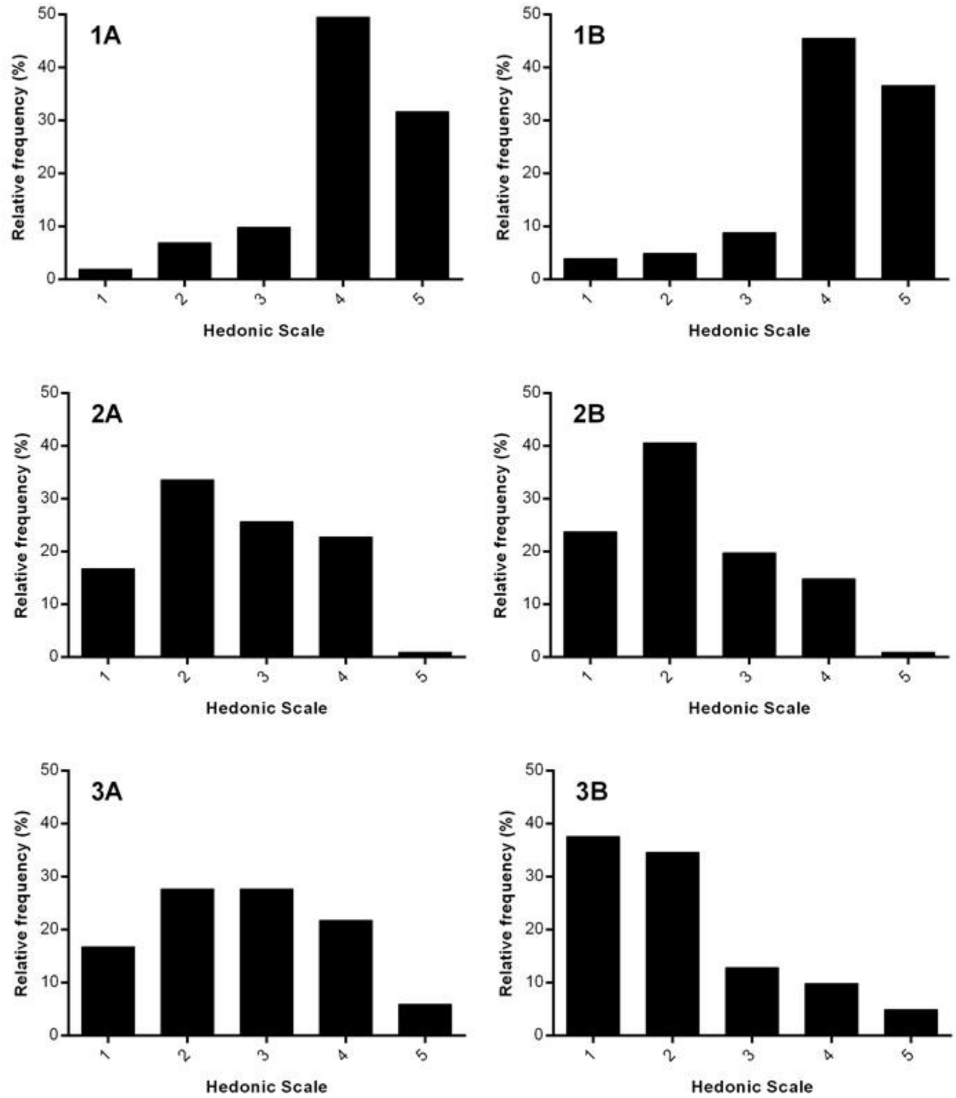
[a,b] Means with different superscript small letter in the same row differ significant by the Cochran’s Q test (P < 0.05).

Figure 1. Acceptability distribution frequency for N poultry breast and those affected by the WS myopathy.



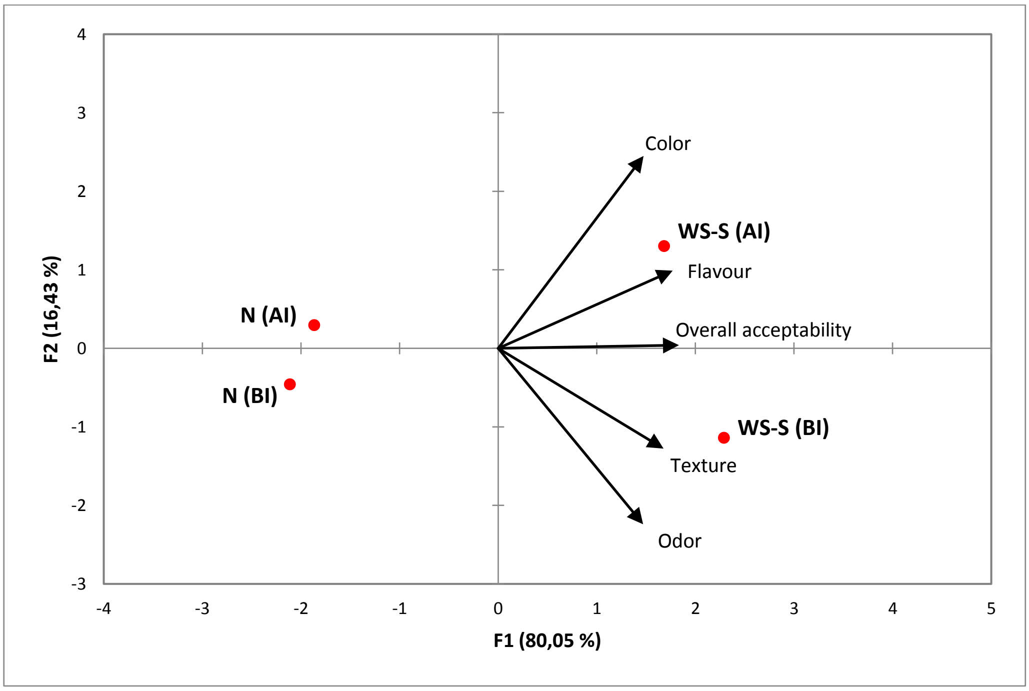
1-Normal; 2-WS-M; 3-WS-S; A-Before information (BI); B-After information (AI). Hedonic Scale: 1- disliked extremely, 2- disliked moderately, 3- neither liked nor disliked it, 4-liked moderately, and 5-liked extremely.

Figure 2. Purchase intent distribution frequency for N poultry breast and those affected by the WS myopathy.



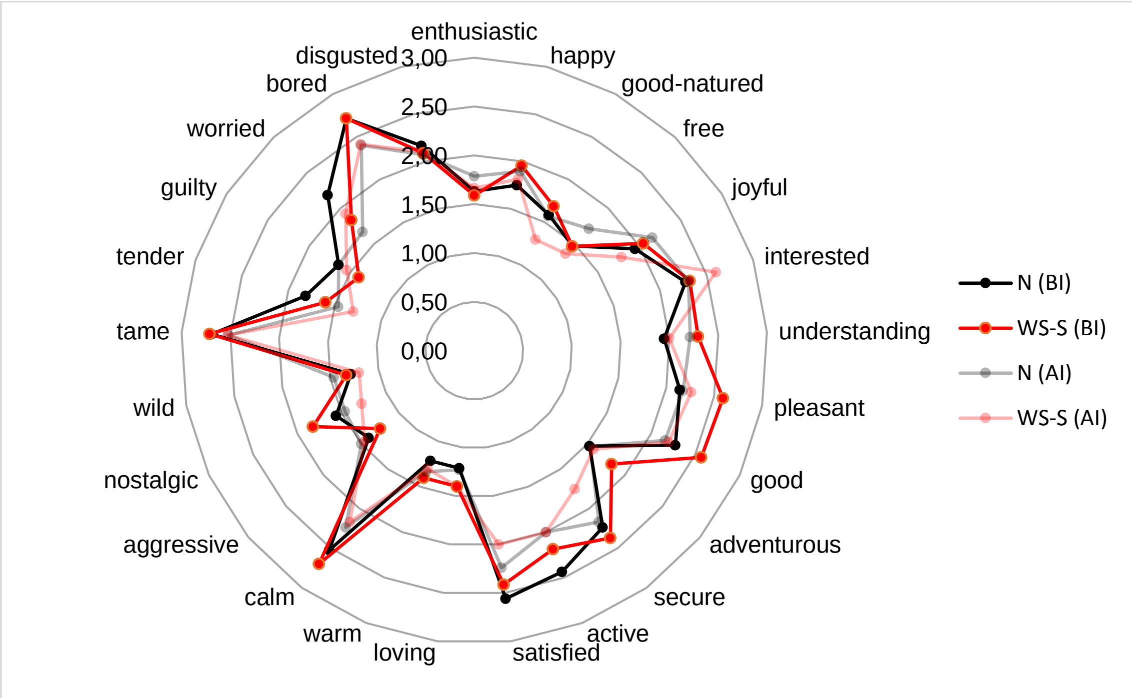
1-Normal meat; 2-WS-M meat; 3-WS-S meat; A-Before information; B-After information. Hedonic Scale: 1- definitely would not buy it, 2- probably would not buy it, 3- may or may not buy it, 4- probably would buy it, and 5- definitely would buy it.

Figure 3. Principal component analysis of quality traits assessed in roasted N and WS-S chicken breasts in both BI and AI scenarios, and projection of the samples in the similarity map.



BI: before information; AI: after information

Figure 4. Rate-all-that-applies assessment of emotions evoked during consumption of roasted N and WS-S chicken breasts, under blind (BI) and informed (AI) scenarios.



BI: before information; AI: after information.

5 CONCLUSÕES GERAIS

Diante dos resultados obtidos nos estudos realizados, pode-se destacar:

i) A ocorrência de miopatias na carne é agravada com o aumento da idade de abate de aves, podendo ser observada em aves desde 4-5 semanas até 65 semanas de idade. Além disso, graus mais leves das miopatias WS, WB e WS/WB são observadas em aves mais jovens. No entanto, aves que não são submetidas ao rápido crescimento em um curto período de tempo (matrizes de corte, idade de abate de 65 semanas) parecem não ser propensas ao desenvolvimento da miopatia WB (isolada ou combinada à WS). Logo, o nível de ocorrência e o grau das miopatias no músculo do peito de frango está diretamente relacionada à idade de abate das aves.

ii) Os resultados confirmam que as miopatias WS, WB e WS/WB apresentam efeito prejudicial sobre as características físico-químicas da carne.

iii) Peitos afetados pelas miopatias WS e WB+WS/WB podem ser distinguidos de peitos Normais (N) e entre si, independentemente da idade de abate, em linha de produção industrial usando o método rápido e não destrutivo de espectroscopia do infravermelho próximo associados à análise multivariada SPA-LDA. Entretanto, esse método não é eficiente para diferenciar peitos WB e WS/WB. Logo, é possível confirmar o uso promissor da técnica NIRS na identificação de miopatias em linhas de processamento industrial.

iv) As proteínas e lipídeos de carnes WS são mais susceptíveis ao estresse oxidativo. Carnes afetadas pelo grau severo de WS apresentam maior depleção dos tióis livres (marcador de oxidação proteica), maior formação de malonaldeído (marcador oxidação lipídica), alisina e bases de Schiff (marcadores de oxidação proteica), e redução da atividade de enzimas antioxidantes endógenas SOD, CAT e GSH-Px.

v) O estudo proteômico revela uma tentativa fracassada de manter a função biológica da fibra muscular e a homeostase redox. O acúmulo de proteínas oxidadas pode ser uma via importante no início da miopatia, pois as proteínas envolvidas no reparo celular e na renovação das proteínas são reguladas em excesso. Os peitos WS apresentam a maior susceptibilidade ao estresse oxidativo, e consequente comprometimento dos processos fisiológicos e metabólicos do músculo.

vi) A avaliação sensorial mostrou que o reconhecimento de peitos estriados é muito baixo entre consumidores espanhóis. Além disso, a aceitabilidade e intenção de compra da carne crua foram significativamente prejudicadas após a informação aos consumidores sobre as causas e consequências da miopatia WS.

vii) O processo de cozimento da carne de frango mascarou a visualização das estrias brancas (WS), e isso resultou na maior preferência, por partes dos consumidores, da carne WS em comparação com a N, em condições não informada de consumo. Por outro lado, quando se considerou a condição informada de consumo, houve uma elevada rejeição a ingestão da carne WS. Além disso, o perfil emocional dos consumidores foi afetado pela conscientização da miopatia WS, uma vez que os consumidores se sentiam menos “bem-humorados”, “amorosos” e “amistosos”, e mais “interessados” e “entusiasmados” ao consumir o peito estriado.

viii) Demostrou-se que o benefício da informação sobre o que os consumidores ingerem comprometem a aceitabilidade, a intensão de compra e as emoções evocadas durante o consumo de peitos de frango estriados.

Sugere-se para estudos futuros a realização de pesquisas que avaliem o uso de outros métodos não destrutivos para distinguir simultaneamente as novas miopatias em carne de aves. Faz-se necessário também o estudo de técnicas rápidas que sejam capaz de distinguir os graus de severidade da miopatias WB, de forma a auxiliar o destino correto das carnes afetadas (processamento e/ou descarte) em atendimento ao Ofício-circular nº 17 de 13 de dezembro de 2019 do Ministério de Agricultura, Pecuária e Abastecimento, visando a utilização da técnica tanto pela indústria quanto por instituições que realizam inspeção de segurança e qualidade. Além disso, recomendam-se estudos sobre estabilidade oxidativa e percepção dos consumidores envolvendo as miopatias Wooden Breast (WB) e Spaghetti Meat (SM).

APÊNDICE

APÊNCICE A – Informativo ao consumidor: Teste de aceitabilidade e intenção de compra

INFORMACION SOBRE LAS PECHUGAS QUE ACABAS DE EVALUAR

Algunas de las pechugas que acabas de evaluar presentan una alteración o defecto reconocido por expertos como "**WHITE STRIPING" o "ESTRIACIONES BLANCAS"** en grados "MODERADO" Y "SEVERO".

Consiste en la aparición de estriaciones blancas en la superficie de la pechuga que corresponde a la acumulación de grasa como síntoma de un proceso de degeneración muscular. **Una pechuga de pollo sano no presenta ese tipo de estrías.**

La causa parece estar relacionada con un crecimiento excesivamente rápido de pollos seleccionados que causa problemas circulatorios, estrés oxidativo y finalmente degeneración grasa y fibrosis.

Estas pechugas se han comprado en un supermercado de Cáceres. **Comer este tipo de carne NO entraña ningún tipo de riesgo para la salud**. Estudios científicos solo han encontrado ligeros cambios en la composición y en el valor nutritivo en comparación con pechugas normales.

Una vez sabiendo esto, por favor **VUELVE A RESPONDER** ¡GRACIAS!

¿CONOCÍAS LA CARNE WHITE STRIPING? ☐ **SI** ☐ **NO**

APÊNDICE B – Ficha de análise sensorial: Teste de aceitabilidade e intenção de compra

CUESTIONARIO

EDAD: 18-23 ☐ 24-31 ☐ 32-41 ☐ 42-51 ☐ 52-61 ☐ 62-71 ☐

GENERO: MASCULINO ☐ FEMENINO ☐

¿ERES COMPRADOR HABITUAL DE CARNE DE POLLO? SI ☐ NO ☐

¿ERES CONSUMIDOR HABITUAL DE CARNE DE POLLO? SI ☐ NO ☐

Marca la opción correspondiente de acuerdo a **TU AGRADO EN RELACIÓN A LA APARIENCIA** de las siguientes pechugas de pollo:

Cod.	Me disgusta mucho 	Me disgusta 	Ni me gusta ni me disgusta 	Me gusta 	Me gusta mucho 

BREVEMENTE: ¿Cuáles son las razones de tu decisión? ____

____

____

Marca la opción correspondiente de acuerdo a tu **INTENCIÓN DE COMPRA** de las siguientes pechugas de pollo:

Cod.	Definitivamente no la compraría	Probablemente no la compraría	Puede o puede que no la compraría	Probablemente la compraría	Definitivamente la compraría

BREVEMENTE: ¿Cuáles son las razones de tu decisión? ____

____

____

APÊNDICE C – Informativo ao consumidor: Teste RATA de emoções e aceitabilidade

INFORMACION SOBRE LAS PECHUGAS QUE ACABAS DE EVALUAR

Una de las pechugas que acabas de avaluar es **NORMAL**, entendiendo que no presenta ninguna alteración aparente de calidad.

La otra pechuga presenta una alteración o defecto reconocido por expertos como **"WHITE STRIPING"** o **"ESTRIACIONES BLANCAS"** en grado "SEVERO".

Estas pechugas se han comprado en un supermercado de Cáceres. **Comer este tipo de carne NO entraña ningún tipo de riesgo para la salud**. Estudios científicos solo han encontrado ligeros cambios en la composición y en el valor nutritivo en comparación con pechugas normales.

Consiste en la aparición de estriaciones blancas en la superficie de la pechuga que corresponde a la acumulación de grasa como síntoma de un proceso de degeneración muscular. **Una pechuga de pollo sano no presenta ese tipo de estrías.**

La causa parece estar relacionada con un crecimiento excesivamente rápido de pollos seleccionados que causa problemas circulatorios, estrés oxidativo y finalmente degeneración grasa y fibrosis.

Una vez sabiendo esto, por favor **VUELVE A CONSUMIR Y A RESPONDER** ¡GRACIAS!

FOTO DE LA PECHUGA "NORMAL"

FOTO DE LA PECHUGA CON "WHITE STRIPING"

APÊNDICE D – Ficha análise sensorial: Teste de aceitabilidade

Nombre: Edad: Consumidor de pollo: Si NO

Comprador de pollo: SI NO

Fecha:

Por favor, deguste la muestra de pollo que se le presenta a continuación en indique la intensidad de la aceptabilidad/agrado de las siguientes características sensoriales marcando en la escala correspondiente:

APARIENCIA (COLOR)

Me disgusta mucho | Me disgusta | Ni me gusta ni me disgusta | Me gusta | Me gusta mucho

OLOR

Me disgusta mucho | Me disgusta | Ni me gusta ni me disgusta | Me gusta | Me gusta mucho

TEXTURA

Me disgusta mucho | Me disgusta | Ni me gusta ni me disgusta | Me gusta | Me gusta mucho

FLAVOR/SABOR

Me disgusta mucho | Me disgusta | Ni me gusta ni me disgusta | Me gusta | Me gusta mucho

GLOBAL

Me disgusta mucho | Me disgusta | Ni me gusta ni me disgusta | Me gusta | Me gusta mucho

APÊNCICE E – Ficha de análise sensorial: Teste RATA de emoções

Nombre:

Edad:

Consumidor de pollo: Si NO

Comprador de pollo: SI NO

Fecha:

Por favor, deguste la muestra de pollo que se le presenta a continuación en indique la intensidad de las emociones que se le presentan en el siguiente listado marcando en la casilla correspondiente:

Emoción	Nada	Ligeramente	Moderadamente	Mucho	Extremadamente
ENTUSIASTA					
FELIZ					
BONDADOSO					
LIBRE					
ALEGRE					
INTERESADO					
COMPRENSIVO					
AGRADABLE					
BUENO					
AVENTURERO					
SEGURO					
ACTIVO					
SATISFECHO					
AMOROSO					
CARIÑOSO					
TRANQUILO					
AGRESIVO					
NOSTÁLGICO					
DESENFRENADO					
INSULSO					
DELICADO					
CULPABLE					
PREOCUPADO					
ABURRIDO					
ASQUEADO					
FELIZ					

ANEXO

ANEXO A – Certificado do Comitê de Ética em Pesquisa envolvendo animais

UNIVERSIDADE FEDERAL DA PARAÍBA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA)

CERTIFICADO

Certificamos que o projeto intitulado "**Incidência e qualidade de peitos de frango com White Striping e Wooden Breast em abatedouros do Nordeste do Brasil**", protocolo nº **031/2017** sob a responsabilidade da pesquisadora Dra. **Marta Suely Madruga** – que envolve a produção, manutenção e/ou a utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 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 aprovado pela Comissão de Ética no Uso de Animais da Universidade Federal da Paraíba (CEUA-UFPB) em reunião de 19/04/2017.

Vigência do Projeto	2017 - 2020
Espécie/linhagem	Ave (Cobb, Ross)
Número de animais	30.000 animais
Idade/peso	40-48 dias (2,5 kg)
Sexo	--------
Origem	Aviários dos Estados da Paraíba, Pernambuco, Rio Grande do Norte e Bahia

IGAGonçalves

Profa. Dra. Islania Giselia Albuquerque Gonçalves
Coordenadora da CEUA-UFPB

ANEXO B – Comprovante de submissão do artigo I

06-May-2020

Dear Dr Estévez,

Your manuscript, "Near-infrared Spectroscopy and Multivariate Analysis to Identify Chicken Breasts Affected by Wooden Breast and White Stripping Myopathies in Brazilian Slaughtering Plants" has been successfully submitted to Journal of Food Science through ScholarOne Manuscripts.

IMPORTANT NOTICE

BY SUBMITTING THIS MANUSCRIPT, YOU WARRANT THAT:
-This article is an original work, has not been published before and is not being considered for publication elsewhere;
-You have obtained permission from the copyright holder to reproduce in the article any material not owned by you, and that you have acknowledged the source(s);
-Your article contains no violation of any existing copyright or other 3rd party right or any material of an obscene, indecent, libelous, or otherwise unlawful nature and that to the best of your knowledge, the article does not infringe the rights of others;
-In the case of a multi-authored article, you have obtained authorization to make these warranties on behalf of all co-authors, and that they have read and agreed to the terms of IFT"s Copyright Assignment Form.

IF YOU CANNOT WARRANT THE ABOVE OR HAVE QUESTIONS ABOUT IFT'S PUBLICATIONS ETHICS POLICY, PLEASE CONTACT US AT JFS@IFT.ORG

The submitting author will receive future communications via e-mail. All authors will be sent the decision letter.

Your manuscript number is JFDS-2020-0739. Please note this number and include it in future communications, and when labelling all electronic files involved with this paper.

The submitting author can track your manuscript by logging on to ScholarOne Manuscripts (https://mc.manuscriptcentral.com/jfs), where the status will be displayed in the submitting author's Author Center.

Thank you for your interest in IFT's Scientific Journals.

Sincerely,

IFT Journals Editorial Office
Institute of Food Technologists®
525 W. Van Buren Street, Suite 1000
Chicago, IL 60607-3830 USA
+1.312.806.8088 Mobile
jfs@ift.org

==
This letter was generated automatically by ScholarOne Manuscripts
https://mc.manuscriptcentral.com/jfs

ANEXO C – Comprovante de submissão do artigo II

*** Automated email sent by the system ***

Dear Dr. Leila Carvalho,

You have been listed as a Co-Author of the following submission:

Journal: LWT - Food Science and Technology
Title: Pinpointing oxidative stress behind the White Striping myopathy: Depletion of antioxidant defenses, accretion of oxidized proteins and impaired proteostasis
Corresponding Author: Mario Estevez
Co-Authors: Leila Carvalho; Josue Delgado; Marta Madruga;

To be kept informed of the status of your submission, register or log in (if you already have an Elsevier profile).

Register here: https://ees.elsevier.com/lwt/default.asp?acw=&pg=preRegistration.asp&user=coauthor&fname=Leila&lname=Carvalho&email=leilamdc@yahoo.com.br

Or log in: https://ees.elsevier.com/lwt/default.asp?acw=&pg=login.asp&email=leilamdc@yahoo.com.br

If you did not co-author this submission, please do not follow the above link but instead contact the Corresponding Author of this submission at mariovet@unex.es.

Thank you,

LWT - Food Science and Technology

ANEXO D – Comprovante de submissão do artigo III

*** Automated email sent by the system ***

Dear Dr. Leila Carvalho,

You have been listed as a Co-Author of the following submission:

Journal: LWT - Food Science and Technology
Title: Consumers Awareness of White-Striping as a Chicken Breast Myopathy Affects Their Purchasing Decision and Emotional Responses
Corresponding Author: Mario Estevez
Co-Authors: Leila Carvalho; Sonia Ventanas; Lary Souza; Marta S Madruga;

To be kept informed of the status of your submission, register or log in (if you already have an Elsevier profile).

Register here: https://ees.elsevier.com/lwt/default.asp?acw=&pg=preRegistration.asp&user=coauthor&fname=Leila&lname=Carvalho&email=leilamdc@yahoo.com.br

Or log in: https://ees.elsevier.com/lwt/default.asp?acw=&pg=login.asp&email=leilamdc@yahoo.com.br

If you did not co-author this submission, please do not follow the above link but instead contact the Corresponding Author of this submission at mariovet@unex.es.

Thank you,

LWT - Food Science and Technology