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WHITE STRIPING AND WOODEN BREAST MYOPATHIES IN BROILER CHICKENS: INSIGHTS INTO POSSIBLE CAUSES AND PREDISPOSING FACTORS, MUSCLE PATHOLOGICAL LESIONS AND IMPLICATIONS ON MEAT QUALITY

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ABSTRACT

White Striping (WS) and Wooden Breast (WB) are two emerging myopathies in broiler hybrid chickens, which are gaining great relevance for the poultry sector, due to the huge economical losses ascribable to the unappealing appearance and impaired nutritional and technological quality of affected fillets. However, the knowledge concerning their aetiology is not clear and the consequences on meat quality is still incomplete. Therefore, the present thesis, which includes four researches, aimed to deepen the knowledge possessed by the scientific community on these defects. The first study investigated the effects of WB myodegeneration on hybrid chickens meat quality and contributed in typifying macroscopic and histologic lesions. To this purpose, weight, cross sectional area (CSA), pH, L*, a*, b* colour values, water-holding capacity, and Warner-Bratzler shear force were determined on 30 normal and 30 WB breasts (*Pectoralis major* muscle). In addition, samples were visually and histologically evaluated. Affected samples were heavier, thicker, paler, and characterized by harder consistency, and higher pH and cooking losses. Macroscopically, the condition affected mainly the cranial portion of the fillet, and was defined by the presence of bulges, petechiae, fluid and clear exudate. Microscopically, muscle fibres with greater CSA were detected, as well as a higher giant fibre prevalence compared to unaffected meat. A relationship between breast weight and WB condition is suggested from the data. The second study evaluated the impact of WS, alone and in combination with WB (WSWB), on the technological quality, mineral, and sensory profile of poultry meat. It emerged that the simultaneous presence of the two myopathies, with respect to the WS lesion individually considered, had a further detrimental effect on pH, yellowness, cooking losses, instrumental and sensory toughness. Mineral contents suggest that a defective ions regulation occurs in lesioned pectoral muscles. The third study investigated the impact of 2 coccidiosis control systems (vaccine vs anticoccidial) and 2 feeding plans (standard energy vs low energy content) on WS prevalence and severity (level 0: no WS; level 1: moderate; level 2: severe) in chicken broiler breasts evaluated at 12, 25 and 51 days. All coccidiosis control systems exerted an ameliorative effect in terms of live weight, breast yield, and whole breast weight; heavier fillets were characterized by higher pH values. White Striping macroscopically appeared at 25 d of age with lesions of moderate severity; at 51 days, total average prevalence was very high, with birds treated with coccidiostat showing the highest incidences of severe lesions. Diet had no effect on overall live performance. The fourth study investigated the role of myowater on WB hardness by integrating low-field nuclear magnetic resonance (NMR) relaxometry with texture analysis (compression test); two muscle conditions (Normal vs WB), four sampling locations (cranial-superficial, cranial-deep, medial- superfi-

cial, medial-deep) and four sampling times (10, 24, 48, 72 h *p.m.*) were considered. As WB affected fillets experience tissue degenerative changes, myowater distribution and properties were found to vary compared to the unaffected counterparts. Moreover, partial water molecules relocalisation toward extramyofibrillar spaces occurs in WB samples during storage, along with an increase in the mobility of water trapped into the myofibrillar matrix. The cranial-superficial layer of breasts exhibited the highest amount of the extramyofibrillar water, and the texture of this muscle part was harder than the deep layers of both cranial and medial portions. Therefore, a positive correlation between muscle hardness and the mobility of water trapped into the myofibrillar matrix was discovered in WB fillets, opening a new hypothesis alternative to fibrosis, to explain the characterising hard consistency of WB, even though the role of myowater on muscle hardness was not fully clarified.

RIASSUNTO

Due miopatie del muscolo pettorale superficiale (*Pectoralis major*) denominate White Striping (WS) e Wooden Breast (WB) stanno assumendo grande rilevanza per il settore avicolo da carne a causa delle ingenti perdite economiche che stanno causando. I danni sono attribuibili all'aspetto visivo e al peggioramento della qualità nutrizionale e tecnologica dei petti affetti. Nonostante ciò, la loro eziologia non è chiara e la conoscenza delle conseguenze sulla qualità delle carni affette è ancora incompleta. Pertanto, la presente tesi di Dottorato, che comprende quattro ricerche, mira ad approfondire le conoscenze possedute dalla comunità scientifica su questi difetti. Il primo studio ha esaminato gli effetti della miodegenerazione del tessuto muscolare del petto di broiler ibridi affetti da Wooden Breast sulla qualità della carne e ha contribuito a tipizzare le lesioni macroscopiche ed istologiche. A questo scopo, i rilievi hanno riguardato il peso, l'area della sezione trasversale (CSA), il pH e il colore (L* a*, b*), la capacità di ritenzione idrica e lo sforzo di taglio. I campioni di petto inoltre sono stati anche valutati visivamente ed istologicamente. I petti affetti da Wooden Breast sono risultati più pesanti, più spessi, più pallidi, più duri al tatto e hanno presentato valori di pH e perdite di cottura più alti. Macroscopicamente, la condizione ha colpito prevalentemente la parte craniale del petto; le lesioni caratterizzanti sono risultate la presenza di globosità, di petecchie, di un essudato fluido e chiaro sulla superficie. Microscopicamente, nel WB si sono osservate fibre muscolari con maggiore CSA, così come una maggiore prevalenza di fibre giganti rispetto ai campioni non affetti. Dai dati ottenuti, si suggerisce una relazione tra il peso del petto e la condizione WB. Il secondo studio ha valutato l'impatto del White Striping sia da solo che in combinazione con il Wooden Breast (WSWB) sulla qualità tecnologica e sui profili minerale e sensoriale dei petti di broiler ibrido. È emerso che la presenza contemporanea delle due miopatie, rispetto al solo WS, ha esercitato un ulteriore effetto peggiorativo sul pH, sull'indice del giallo, sulle perdite di cottura, sulla durezza strumentale e sensoriale. Il profilo minerale ottenuto suggerisce uno squilibrio ionico nelle fibre muscolari dei petti affetti. Il terzo studio ha valutato l'impatto di due metodi di controllo della coccidiosi (vaccino vs coccidiostatico) e di due piani di alimentazione (standard vs basso contenuto di energia) sulla prevalenza e la gravità del WS (livello 0: assenza, livello 1: moderato, livello 2: grave) in petti di broiler ibridi esaminati a 12, 25 e 51 giorni di età. Entrambi i sistemi di controllo della coccidiosi hanno esercitato un effetto migliorativo su peso vivo, resa in petto e peso del petto; i petti più pesanti sono stati caratterizzati da valori di pH più elevati. Il WS è comparso macroscopicamente a 25 giorni di età con lesioni di moderata gravità; a 51 giorni la prevalenza media totale è stata molto elevata, con il gruppo trattato con coccidiostatico che ha evidenziato la maggior incidenza di lesioni gravi. La dieta non ha avuto alcun effetto sulle prestazioni produttive.

Il quarto studio ha esaminato il ruolo dell'acqua contenuta nel muscolo pettorale del broiler ibrido sullo sviluppo della tipica durezza del WB, integrando analisi di risonanza magnetica nucleare (NMR) e test di compressione. Come fattori, sono stati considerati due condizioni muscolari (Normale vs WB), quattro punti (craniale-superficiale, craniale-profondo, mediano-superficiale, mediano-profondo) e quattro tempi di conservazione (10, 24, 48, 72 h *p.m.*). Poiché i petti WB esibiscono alterazioni degenerative dei tessuti, di conseguenza la distribuzione e le proprietà delle molecole d'acqua presenti nel muscolo sono risultate diverse rispetto ai petti normali. Inoltre, nel corso della conservazione *post mortem*, i petti Wooden Breast hanno evidenziato una parziale redistribuzione dell'acqua a favore della frazione extramiofibrillare, unitamente ad una maggiore mobilità della frazione d'acqua intrappolata nella matrice miofibrillare. La porzione craniale- superficiale dei petti ha evidenziato una maggior quantità di acqua extramiofibrillare ed una consistenza più dura rispetto agli strati profondi di entrambe le parti craniale e mediale del muscolo. Pertanto, è emersa nel WB una correlazione positiva tra la durezza muscolare e la mobilità dell'acqua intrappolata nella matrice miofibrillare, aprendo una nuova ipotesi alternativa alla fibrosi per spiegare la caratteristica durezza del WB, anche se il ruolo dell'acqua sulla durezza muscolare non è stato ancora completamente chiarito.

CHAPTER 1

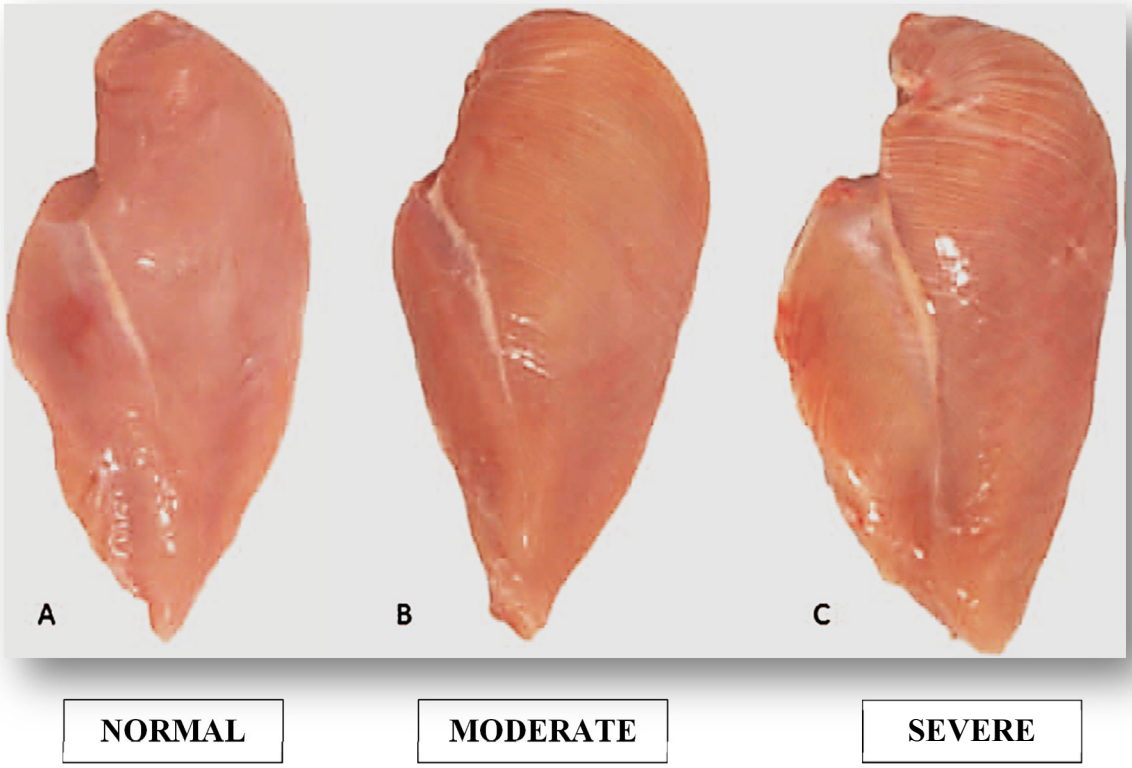
General introduction

During the last decades, poultry meat production and consumption have increased rapidly worldwide, thanks to many positive aspects that make poultry meat an attractive product: low and competitive price, absence of cultural or religious obstacles, easiness to handle and to process, and healthy dietary and nutritional properties (Barroeta, 2006; Valceschini, 2006; Givens, 2009). Therefore, to fulfil the ever-increasing demand for chicken meat, poultry supply chain has been enforced to improve growth rate, feed efficiency and breast meat yield of broilers using high-energy diets and intensive selection of genotypes exhibiting faster growth and higher breast yields (Petracci et al., 2013b). On one hand, challenging birds to reach high body and breast weights within a short rearing period permitted to reach a greater production output. On the other hand, rapidly growing strains exhibited a substantial onset of breast myopathies (deep pectoral myopathy, pale, soft and exudative meat, toxic myopathy, nutritional myopathy, intramuscular connective tissue immaturity) during the last decades, which resulted in profound negative impact on meat quality issues (Petracci & Cavani, 2012). Only recently, global attention has been drawn to other two emerging defects affecting the breast (*Pectoralis major* muscle) of chicken hybrids: one is the presence of white striations on the muscle surface, referred as "White striping" (WS) (Kuttappan et al., 2012a); the other, characterised by the hard consistency of fillets, has been called "Wooden Breast" (WB) (Sihvo et al., 2014). These conditions are gaining great relevance for the poultry sector, due to the huge economical losses ascribable to the unappealing appearance (Kuttappan et al. 2012b; Sihvo et al., 2014). The first investigations indicate impaired nutritional and technological traits of affected fillets, along with high percentages achieved by WS and WB (Tables 1.1-1.3) both at slaughter and under experimental conditions. The knowledge concerning their aetiology is still incomplete, even though the majority of the results obtained so far indicate that selection for broiler traits (fast growth rate and high breast yield) is the most responsible factor.

Wooden Breast and White Striping visual appearance and histological traits

White Striping has been studied since 2009, and macroscopically described as the presence of white superficial striations parallel to the muscle fibres direction (Bauermeister et al., 2009; Kuttappan et al., 2009, 2012c, 2013b) mainly occurring in raw chicken *Pectoralis major* muscle and thighs (Kuttappan et al., 2013b). The nature of these white striations, which give to the affected fillets an anomalous marbled impression, is not clear and might be a manifestation of mineralization, or the infiltration of collagen or fat (Kuttappan et al., 2013b). According to Kuttappan et al. (2012c), affected fillets could be scored based on the condition severity, as moderate (MOD) or severe (SEV), respectively when white lines are generally < 1 mm thick but easily visible, and when white lines are > 1 mm thick and widespread on the surface (Figure 1.1). The condition is more severe toward the thicker skin-side cranial portion of the *Pectoralis major* muscle, whereas the skin-side caudal end and the bone-side of the breast are less affected by gross lesions (Kuttappan et al. 2013b).

Figure 1.1 White Striping severity degrees (source: Kuttappan et al., 2013a).



A remarkably hard consistency at palpation, together with the presence of bulges and a notably paleness, macroscopically characterizes Wooden Breast (WB) affected *Pectoralis major* muscles. Moderate cases exhibit a prominent firm bulge on the cranial end of the muscle, while severely impacted breasts are recognisable by hard consistency widespread overall the fillet (Sihvo et al. 2014). The same authors also detected other macroscopic lesions or "descriptors" that are frequently visible on the affected surface in addition to the above-mentioned damages: a mainly fluid and clear exudate, petechiae or small haemorrhages, and white superficial stripes of 0.5 to 3 mm thickness running parallel to the myofibers. (Figure 1.2). The presence of extended areas with visibly poor cohesive fibre bundles beneath the damaged areas was concomitant to the superficial lesions (Sihvo et al., 2014) (Figure 1.3).

Figure 1.2 WB macroscopic superficial lesions (source: Puolanne & Dalle Zotte, 2014).

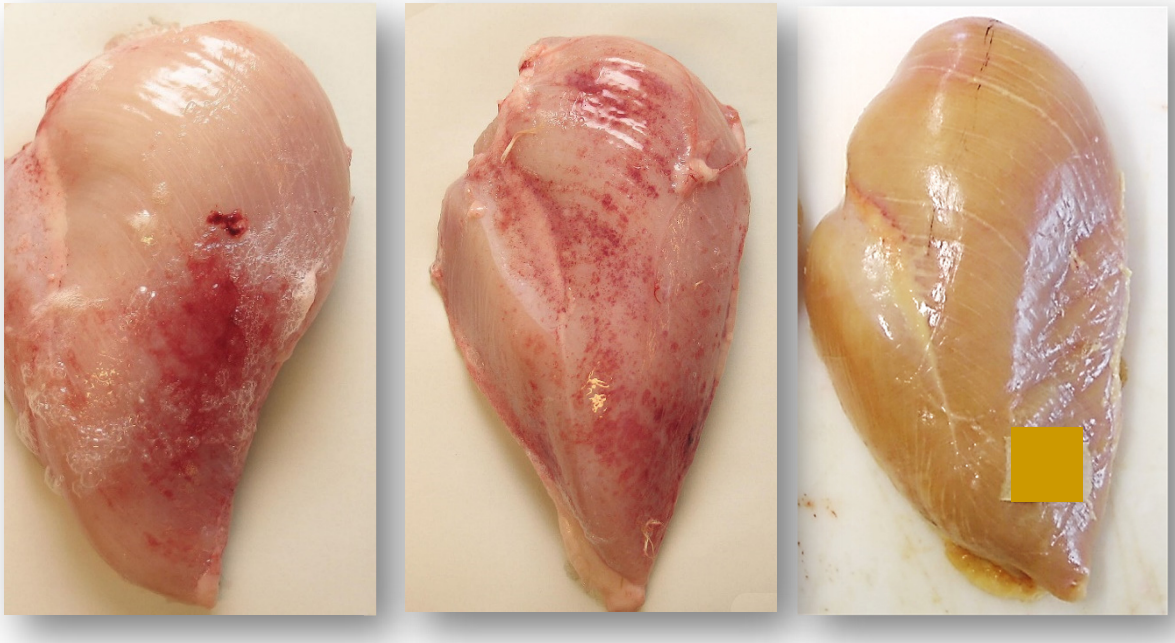
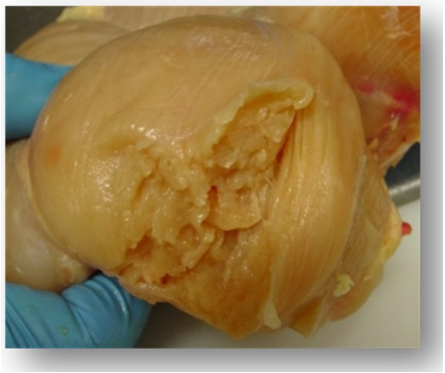


Figure 1.3 Poor cohesive fibre bundles (source: Puolanne & Dalle Zotte, 2014).



Since the macroscopic co-occurrence of White Striping and Wooden Breast within the same fillet was described in the literature (Sihvo et al., 2014; Kindlein et al., 2015; Mudalal et al., 2015; Petracci et al., 2015), it is not surprising that the two conditions exhibit overlapping histological traits (Sihvo et al., 2014); therefore, a common causative basis has been hypothesized for the two myopathies (Mudalal et al., 2015). Indeed, both Wooden Breast and White Striping are characterised by degenerative myopathic lesions with the replacement of the damaged tissue by fibrosis and lipidosis as reparative responses. Tissue sections indicate a continuous exposure to the causative insult, as histology is characterized by the concomitant occurrence of both acute and chronic (polyphasic) changes, with the chronicity of microscopic lesions increasing as the severity of White Striping and Wooden Breast increases (Kuttappan et al., 2013b). As revealed by the histologic cross sections, myofibres architecture is altered in affected muscular tissue; indeed, different cross-sectional areas (giant and small cells within the same stain), loss of the normal polygonal profile (rounded fibres), loss of cross striations, nuclei internalization, and splitted cells were detected. These changes were accompanied by myofibres necrosis and phagocytosis, along with regeneration (presence of fibroblasts) and inflammatory cells infiltration (macrophages, heterophils, lymphocytes) around the degenerated fibres (Kuttappan et al., 2013b; Sihvo et al., 2014). As firstly described by Sihvo et al. (2014) in Wooden Breast, affected muscle experiences moderate or severe endomysium (interstitium), perimysium and epimysium thickening due to the accumulation of different types of connective tissue (fibrosis), which is intermixed with the infiltration of inflammatory cells (heterophils, macrophages and lymphocytes). Specifically, variable amounts of loose connective tissue, granulation tissue or collagen-rich connective tissue were detected to separate myofibres. At the same time, perimysium was found to exhibit moderate to severe oedema and proliferation of loose connective tissue separating fibre bundles, while epimysium seemed to be thickened by loose connective possessing abundant amorphous extracellular material and variable amounts of collagen fibres (Sihvo et al., 2014).

Morphometric traits revealed that *Pectoralis major* muscles affected by all these abnormalities experience altered physical growth pattern with respect to the Normal ones. Indeed, from the studies of Mudalal et al. (2015) it is evident that insulted fillets are globally heavier (N: 245 g vs WS: 305 g; WB: 299 g; WSWB: 318 g; $P < 0.001$) and thicker at the cranial portion than unaffected breasts (N: 38.1 mm vs WS: 45.7 mm; WB: 43.9 mm; WSWB: 45.7 mm; $P < 0.001$). Moreover, Wooden Breast, either alone or in combination with White Striping, emerged among all groups for fillets with the thickest caudal end (N: 8.2 mm and WS: 8.7 mm vs WB: 11.0 mm and WSWB: 11.6 mm; $P < 0.001$) and the hardest consistency at raw state (N: 2.02 kg and WS: 2.28 kg vs WB: 4.02 kg and WSWB: 3.33 kg; $P < 0.001$). Overall, the findings of Mudalal et al. (2015) indicated that the heavier weight of the insulted fillets did not result in a simultaneous greater length and width; therefore, according to the previous observations of Lubritz (1997) and Brewer et al. (2012), it was postulated that an increase in breast weight mostly affects breast thickness rather than the other morphometric traits.

At present, little information is available on the *in vivo* clinical symptoms ascribable to the occurrence of these emerging myopathies, and no standardized methods have been developed to detect their presence on live chickens. Nevertheless, Velleman & Clark (2015) reported the possibility to detect the typical hard consistency of Wooden Breast by palpation on alive birds, discriminating between rubber elastic (WB) and flexible (normal) consistencies.

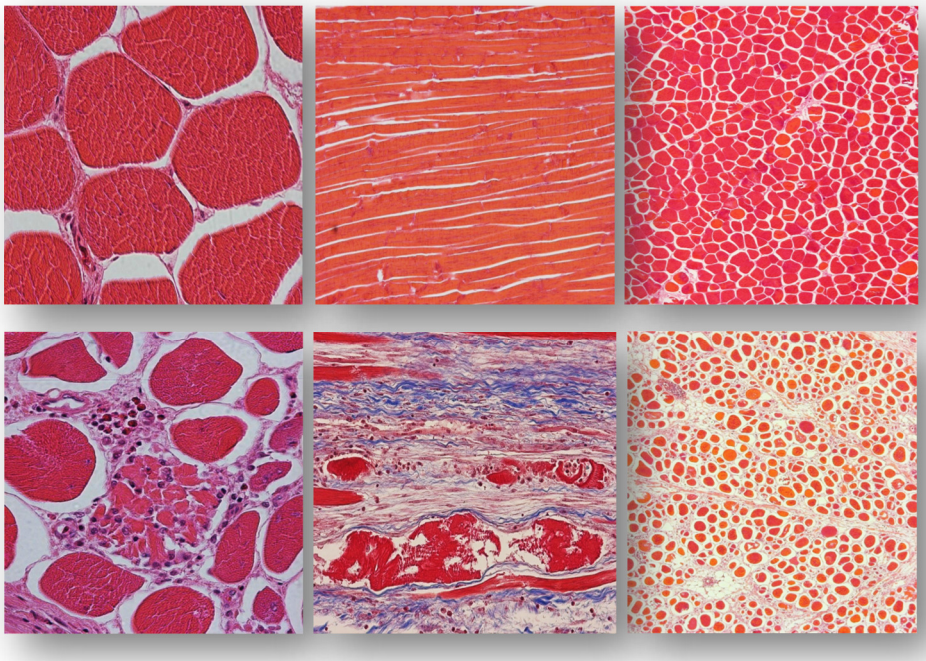


Figure 1.4 Normal (A) vs WB and WS histological traits (B):

- **B1**: cell degeneration + accumulation of inflammatory cells;
- **B2**: fibrosis;
- **B3**: separated, small, round cells

Source: Sihvo (2012)

Table 1.1 White Striping incidence at slaughter age.

References	Hybrid	Occurrence (%)	BW[1] (kg)	Age (d)[2]	Sex	Conditions
Kuttappan et al. (**2012**)	Cobb 500	➢ TOT: 63.6 ➢ MOD: 58.5 ➢ SEV: 5.10	-	54	-	Commercial USA
Petracci et al. (**2013**)*	High yield	➢ TOT: 12.0 ➢ MOD: 8.90 ➢ SEV: 3.10	2.75	45-54	-	Commercial Italy
Ferreira et al. (**2014**)	Cobb 500	➢ TOT: 9.84 ➢ MOD: 7.38 ➢ SEV: 2.46	3.20	42	M	Commercial Brazil
Lorenzi et al. (**2014**)*	Medium Heavy	➢ TOT: 43.0 ➢ MOD: 36.8 ➢ SEV: 6.20	3.0 4.2	41-50 50-58	M+F M	Commercial Italy
Bailey et al. (**2015**)	High yield Moderate yield	49.6 14.5	2.33 1.91	42 32	-	Commercial UK
Kindlein et al. (**2015**)*	Cobb 500	➢ TOT: 82.7 ➢ MOD: 38.8 ➢ SEV: 43.9	-	35 42	-	Experimental Brazil
Russo et al. (**2015**)*	Medium Heavy	➢ TOT: 76.4 ➢ MOD: 56.9 ➢ SEV: 19.5	2.60 3.60	46 55	F M	Commercial Italy

* = average occurrence percentages between different slaughter age

Table 1.2 Wooden Breast incidence at slaughter age.

References	Hybrid	Occurrence (%)	BW[1] (kg)	Age (d)[2]	Sex	Conditions
Bailey et al. (**2015**)	High yield Moderate yield	3.19 0.16	2.33 1.91	42 32	-	Commercial UK
Kindlein et al. (**2013**)	Cobb 500	32.1 89.5	2.62 3.37	35 42	-	Experimental Brazil

Table 1.3 WSWB incidence at slaughter age.

References	Hybrid	Occurrence (%)	BW[1] (kg)	Age (d)[2]	Sex	Conditions
Bailey et al. (**2015**)	High yield Moderate yield	3.19 0.16	2.33 1.91	42 32	-	Commercial UK
Kindlein et al. (**2013**)	Cobb 500	32.1 89.5	2.62 3.37	35 42	-	Experimental Brazil

[1] = body weight
[2] = days of age

Impact of emerging myopathies on meat quality

1. Proximate composition and nutritional quality of affected breasts

Chicken breast meat is considered not only as a high-quality meat facing the modern consumer demand for its low content of lipids, cholesterol and sodium. It is also defined as "functional food" providing compounds favourable for human health: high polyunsaturated fatty acids, bioactive peptides, essential amino acids and vitamins (Cavani et al., 2009; Gibbs et al., 2010; Ryan et al., 2011). However, the studies conducted so far reported that the occurrence of White Striping and Wooden Breast brings about great detrimental changes ascribable to muscular tissue degeneration and reparative responses (fibrosis and lipidosis), which severely compromise meat nutritional value (Mudalal et al., 2014; Petracci et al., 2014, 2015). Indeed, according to Petracci et al. (2015), Wooden Breast in combination with White Striping was found to lower total protein (N: 23.4% vs WSWB: 18.5%; $P \leq 0.05$) and ash contents (N: 1.46% vs WSWB: 1.19%; $P \leq 0.05$). These changes were coupled with higher moisture (N: 74.6% vs WSWB: 76.8%; $P \leq 0.001$), intramuscular fat (N: 0.79% vs WSWB: 1.79%; $P \leq 0.001$) and collagen percentages (N: 1.16% vs WSWB: 1.35%; $P \leq 0.05$). White Striping occurrence leads to a similar impairment of meat chemical composition, and it is worthy to be noticed, that this worsening effect is not confined to the severe manifestations of the myopathy. Indeed, it also involves the moderate WS cases, which are not downgraded but are still purchased as fresh meat (Petracci et al., 2014). Compared to the Normal fillets, protein content is reduced as the severity goes from moderate (MOD) to severe (SEV) White Striping (N: 22.9% vs MOD: 22.2% vs SEV: 20.9%; $P < 0.001$); lipids (N: 0.78% vs MOD: 1.46% vs SEV: 2.53%; $P < 0.001$) and collagen (N: 1.30% vs MOD: 1.37% vs SEV: 1.43%; $P < 0.001$) follow the opposite trend (Petracci et al., 2014). Because of an impaired proximate composition, lipidosis and fibrosis also carry the worsening of some estimated nutritional indexes both in moderate and severe WS (Petracci et al., 2014). Indeed, affected fillets are more energetic (N: 421 vs SEV: 450 kJ/100 g; $P = 0.009$), and the energy contribution from fat augments (N: 29.5 vs MOD: 54.9 vs SEV: 95.6 kJ/100 g; $P < 0.001$) as well as the fat/protein ratio (N: 0.03 vs MOD: 0.07 vs SEV: 0.19; $P < 0.001$), to the detriment of the contribution from protein (N: 392 vs MOD: 381 vs SEV: 355 kJ/100 g; $P < 0.001$). Obviously, the fibrotic response in White Striping is responsible of a higher collagen/protein ratio in comparison to normal (N: 5.72 vs MOD: 6.19 vs SEV: 6.73; $P < 0.001$) (Petracci et al., 2014). This implies a reduced protein quality, as collagen is characterized by low digestibility and deficiency of some essential amino acids with respect to myofibrillar and sarcoplasmic proteins (Young & Pellett, 1984; Boback et al., 2007). As a result of the degeneration processes, the electrophoretic analysis of Normal and lesioned fillets revealed that the presence of myopathic changes not only leads to a decline of the total con-

tractile (N: 85.5 vs WS: 65.3 mg/g of meat, $P < 0.001$) and sarcoplasmic proteins (N: 52.0 vs WS: 44.8 mg/g of meat; $P < 0.01$) in favour of a higher collagen content, but that it also exerts a noteworthy effect on their patterns (Mudalal et al., 2014). In particular, severely WS affected breasts had lowered actin (42kDa), LC1 slow-twitch light chain myosin and LC3 fast-twitch light chain myosin concentrations; this impairment in protein quantity and quality results in defective protein functionality (reduced ability to bind and hold water, reduced protein solubility and emulsifying abilities). As for sarcoplasmic proteins, White Striping reduces the concentrations of several enzymes involved in glycolytic-gluconeogenesis pathways (expressed as mg/g meat): glycogen phosphorylase (GP), pyruvate kinase (PK), phosphoglucose isomerase (PGI), enolase, aldolase (ALD), glyceraldehyde phosphate dehydrogenase (GAP), lactate dehydrogenase (LDH) and phosphoglycerate mutase (PGAM). These discordances from the normal situation were ascribed to the loss of sarcolemmal functionality occurring in part of the myofibres belonging to myopathic tissues, and to the resulting leakage of sarcoplasmic fluids and proteins. It might be that defective enzymatic patterns could also be interpreted as signs of an impaired ability of the muscle tissue to satisfy its energy demand under pathological conditions (Mudalal et al., 2014; Sihvo et al., 2014).

According to Kuttappan et al. (2012b), severely white-striped fillets possess a higher fat content (N: 3.03 g/100 g vs WS-SEV: 5.56 g/100 g; $P < 0.05$) and more unsaturated than the normal counterparts do. Indeed, the level of saturated fatty acids (SFA) was found to be lower in severe WS (N: 32.1% vs WS: 30.0%; $P < 0.05$), whereas the same affected group had a greater overall proportion of monounsaturated fatty acids (MUFA). The most important changes between White Striping and normal breasts concerned the polyunsaturated (PUFA) fraction profile, despite White Striping and normal fillets exhibited the same PUFA overall contents. Indeed, greater amounts of linoleic (C18:2n-6) (N: 22.3% vs WS: 24.5%; $P < 0.05$) and linolenic (C18:3n-3) (N: 1.19% vs WS: 1.59%; $P < 0.05$) fatty acids were detected in WS meat than the unaffected counterpart. Besides possessing higher linolenic and linoleic acids proportions, the same lesioned breasts owned also greater Δ^9- desaturases and elongase activities. Nevertheless, severely white striped breasts exhibited lower amounts of eicosapentaenoic (EPA, C20:5n-3), docosapentaenoic (DPA, C22:5n-3) and docosahexaenoic (DHA, C22:6n-3) essential fatty acids. Previously, Tuazon & Henderson (2012) positively correlated a higher linoleic acid with sarcolemmal damage and oxidative stress; therefore, a relationship between muscle tissue lesions and muscle fatty acid profile might be postulated, even though the source of variations between lesioned and unaffected fatty acid profiles has to be clarified (Kuttappan et al., 2012b). Besides the above-mentioned nutritional quality impairment, breasts affected by Wooden Breast and White Striping showed higher calcium (N: 7.8 mg/100 g vs WSWB: 11.3 mg/100 g; $P < 0.05$) and sodium (N: 37.8 mg/100 g vs WSWB: 75.1 mg/100 g; $P < 0.001$) levels (Petracci et al.,

2014). An altered mineral profile suggests that a defective cations regulation associated with the myopathic changes (Sandercock et al., 2009) is occurring in White Striping and Wooden Breast, and contributes in lowering the nutritional value of a meat appreciated by the consumers for its healthy nutritional profile.

2. Technological quality of affected fillets

2.1. Consumer acceptance, pH and colour traits

The presence of myopathic lesions on the raw breast surface decreases the positive attitude of the consumer toward chicken meat; in this regard, the acceptance and hedonic scores achieved by the moderately and severely white-striped fillets was notably lowered compared to normal ones, and over 50% of the consumers affirm that they would probably or definitely not buy affected fillets for their fatty, marbled appearance (Kuttappan et al., 2012c). However, beyond an impaired visual appearance of raw fillets, the impact of White Striping and Wooden Breast on the sensory properties of cooked chicken meat is not known to date. As breasts can not be offered for fresh retailing, producers are therefore enforced to manufacture processed products (Kuttappan et al., 2012c; Petracci et al., 2013a), even though these abnormalities considerably impair the main technological properties and therefore, meat processing aptitude.

Overall, a slight but significant difference was detected concerning pH values between White Striping and Normal breasts (on average, N: 5.81 vs WS: 5.88; $P < 0.05$) (Mudalal et al., 2014; Trocino et al. 2015). Considering White Striping severity, the pH values exhibit a raising trend as the WS degree becomes severe (N: 5.86 and WS-MOD: 5.88 vs WS-SEV: 5.95; $P \leq 0.05$) (Petracci et al., 2013a). As for Wooden Breast, there is no consensus on its effect on pH; indeed, in some studies, affected fillets and their Normal counterparts exhibited similar pH values that globally did not exceed 5.87 (Mudalal et al., 2015; Trocino et al. 2015). From the studies conducted so far, it emerged that the simultaneous presence of White Striping and Wooden Breast on the same fillets not only aggravates the pH increase respect to the Normal condition (N average pH: 5.86), but also respect to WS and WB singularly detected. Indeed, both moderate and severe WSWB groups exhibited pH values which were on average not below 6.04 at 24h *post mortem* (Mudalal et al., 2014; Petracci et al., 2015). In impacted breasts, a higher ultimate pH could be ascribable to the impaired glycolytic potential (Le Bihan-Duval et al., 2008), which does not permit correct *post mortem* lactate production and meat acidification. This mechanism is substantiated by the strong negative correlation existing between glycogen store (glycolytic potential) and breast muscle weight, and on the other hand, by

the strong positive correlation between breast weight and high ultimate pH of meat (Le Bihan-Duval et al., 2008). In other words, it means that fillets having a greater size, as WS, WB and WSWB, could exhibit a reduced glycolytic potential, resulting in higher ultimate pH.

Colour values (L*a*b*) are also notably changed by the occurrence of emerging myopathies. Indeed, despite their high pH values, a peculiar trait of WB fillets consist in a higher lightness (L*) than either the Normal (N: 50.7 vs WB: 54.2; $P < 0.001$), the WS (WS: 54.9 vs WB: 57.0; $P < 0.001$) and the WSWB fillets (WSWB: 55.2 vs WB: 57.0; $P < 0.001$), conferring to Wooden Breast affected meat the featuring paleness (Mudalal et al., 2015). As for redness index, White Striping occurrence modified a* index when compared to the Normal meat (Petracci et al., 2013a), and the change was already detectable in the moderate degree (N: 1.21 vs WS-MOD: 1.50 and WS-SEV: 1.77; $P \leq 0.05$). This redder colour in lesioned breasts was hypothesized to derive from an up-regulation of myoglobin occurring in myopathic insult. This myoglobin overexpression might be concomitant to the "fiber-type switching"phenomenon, that is the conversion from Type II (fast twitch) fibres into Type I (slow twitch) fibres occurring under pathological changes as adaptative response to environmental cues (Pette & Staron, 2001; Mutryn et al., 2015). Previous studies indicated an increase in yellowness index ascribable to a higher percentage of intramuscular fat (Kuttappan et al., 2013a), which confers to the fillets a more fatty or marbled appearance, less appealing for the consumers (Kuttappan et al., 2012c). To this respect, the highest yellowness characterized Wooden Breast fillets when compared either to Normal, White Striping and WSWB groups (WB: 3.27 vs N: 2.72, WS: 2.70 and WSWB: 2.64; $P \leq 0.05$) (Mudalal et al. (2015), even though also the severe degree of White Striping was significantly more yellow than the normal condition (N: 2.37 vs WS-SEV: 3.16; $P \leq 0.05$) (Petracci et al. 2013a).

2.2. Muscle structure and texture

From the observations of Wooden Breast and White Striping histological stains, it clearly emerged that fillets experienced changes in muscle structure, which result from the degenerative processes occurring under myopathic conditions (Kuttappan et al., 2013a; Sihvo et al., 2014). Some trials aimed at evaluating the effects of WS and WB on raw and cooked breast meat hardness, performing compression tests on both raw and cooked meat and carrying out shear force measurements on cooked samples with Allo-Kramer shear force (AKSF). Raw meat samples that were collected from the caudal end of Wooden Breast and WSWB affected fillets and compressed at 40% of their initial height were found to possess higher compression values than their Normal and WS counterparts (N: 2.00 and WS: 2.28 kg vs WB: 4.10 and WSWB: 3.33 kg; $P < 0.001$) (Mudalal et al., 2015).

Even though some cases of hard Wooden Breast consistency were detected without an increased deposition of collagen in broiler chickens at slaughter age (Sihvo et al., 2014), reparative fibrosis has been considered the major reason explaining the hard consistency characterising raw Wooden Breast affected fillets so far. Indeed, an abnormal accumulation of extracellular matrix (ECM) and of its components collagen and proteoglycans, which could play a role in muscle stiffness, usually occurred in lesioned tissues (Kolakshyapati, 2014). In detail, collagen is the predominant fibrous macromolecule deposited in epimysium and perimysium (McCormick, 1994; Nishimura et al., 1996), which provides tensile strength to the muscle tissue (Velleman et al. 1997), while proteoglycans (PG) are essential in maintaining a normal tissues structure and their optimal mechanical function (Eggen et al. 1994; Velleman et al., 1997). Interestingly, Velleman & Clark (2015) noticed different collagen distribution and arrangement between two chicken genotypes, which were both exhibiting fibrosis and Wooden Breast. This discrepancy was ascribable to a different expression of the ECM proteoglycan decorin regulating collagen crosslinking; where overexpressed, decorin was found to lead to an extensively more tightly packed fibrils and thus to a tougher meat. As different genotypes manifested a different expression of decorin, it was suggested that WB fibrosis might vary in composition, severity and structure, thus differently affecting meat quality (Velleman & Clark, 2015).

As for the effect of myopathies on cooked meat shear force, there is no consensus among the different studies available in the literature. Indeed, Mudalal et al. (2015) stated that both cooked White Striping and Wooden Breast fillets were as hard as their normal counterparts. Petracci et al. (2013a) reported different findings. In that study, the severe degree of White Striping evidenced even a softer consistency than both normal and moderately affected meat after cooking (N: 4.91 kg and WS-MOD: 4.41 kg; WS-SEV: 3.69 kg; $P \leq 0.05$), which was ascribable to the poor cohesion between the fibre bundles. Globally, the discrepancies observed between raw and cooked fillets texture suggest that Wooden Breast tactile and instrumental hardness only concern the raw state, as it is not maintained in cooked meat as collagen molecules are composed by thermally labile cross- links and solubilize at temperatures between 53 and 63°C (Martens et al., 1982).

2.3. Technological quality of affected fillets: water holding capacity

Literature indicates a pronounced impairment of water holding capacity in WoodenBreast and White Striping *Pectoralis major* muscles, which was expressed by the estimation of liquid retention during refrigeration (measured as drip loss) and after cooking (measured as cooking loss). In detail, as demonstrated by Petracci et al. (2013a), both moderate and severe White Striping did not impact on meat dripping (N: 1.04; WS-MOD: 1.08 and WS-SEV: 1.06; $P > 0.05$). Differently, Wooden Breast

manifested greater drip losses percentages when compared to normal (N: 0.93 vs WB: 1.19%; $P \leq 0.05$), to white striped (WS: 0.72 vs WB: 1.19%; $P \leq 0.05$) and even to WSWB fillets (WSWB: 1.03 vs WB: 1.19; $P \leq 0.05$) (Mudalal et al., 2015). Probably, in spite of a high pH, an extensive loss of membrane integrity, as well as the presence of a thin layer of exudate covering breasts surface (Sihvo et al., 2014) have been speculated to be promoting events for the WB drip losses increase (Mudalal et al., 2015). As for WSWB fillets, Mudalal et al. (2015) noticed that the drip losses of WSWB group were similar to those exhibited by the unaffected counterparts; it is likely that the very high pH value of this group (above 6.00) mitigates the fluid leakage ascribable to the loss of membrane integrity.

As for cooking losses, compared to the normal counterparts, fillets loose a higher amount of cooking juices as the severity of White Striping arose (N: 21.3% vs WS-MOD: 23.2% vs WS-SEV: 26.7%; $P \leq 0.05$) (Petracci et al., 2013a) and when Wooden Breast occurs (N: 21.6 vs WB: 28.0% and WSWB 29.5%; $P \leq 0.05$) (Mudalal et al., 2015). It is worthy to be noticed that, with respect to White Striping, the occurrence of Wooden Breast or both White Striping and Wooden Breast within the same fillet sharpen the water retention impairment observed for cooked meat (N: 21.6 vs WS: 24.7 vs WB: 28.0 and WSWB: 29.5%; $P \leq 0.05$) (Mudalal et al., 2015). This observation on meat quality, together with the similar pathological changes detected at histological level by Kuttappan et al. (2009) and Sihvo et al. (2014), made same authors postulate that WS and WB could be the same abnormality, with the latter developing at a later stage and carrying a more severe muscle damage (Mudalal et al., 2015). In lesioned breasts, because of myodegeneration and reparative responses, a massive decline of total and myofibrillar proteins (mainly of myosin and actin) occurs (Mudalal et al., 2014) in favour of collagen. Thus, protein functions such as water holding capacity are notably reduced (Petracci et al., 2015), even though the collagen found in young birds muscles can swell, solubilize, gelatinize and thus retain an amount of water during cooking (Palka, 1999; Tornberg, 2005). The reduction of water holding capacity in these emerging myopathies is independent from pH, as lesioned fillets possessed higher pH and a reduced ability to bind and retain liquids at the same time. On the contrary, the impairment of myofibrillar proteins quantity and functionality are the primary responsible for the higher cooking losses of impacted fillets, along with the myofibers shrinking, which gives rise to large gaps both within myofibrils (intramyofibrillar spaces) and between myofibers (extramyofibrillar spaces), thus rising cooking losses (Pearce et al., 2011).

2.4. Quality traits of processed products

From several studies, it emerged that the presence of myopathic lesions significantly impairs meat processing aptitude and quality properties of processed products; in particular, literature clearly proved that Wooden Breast abnormality, irrespective of the simultaneous occurrence of white striations, leads to a more negative quality implications than White Striping only. The reduced meat water holding and binding ability observed in non-marinated insulted fillets also characterized the marinated counterparts, as a result of the dramatic reduction of total crude protein and in particular of the myofibrillar ones (Mudalal et al., 2015).

According to Mudalal et al. (2015), marinade uptake is lower in white striped fillets (N: 13.2% vs WS: 9.33%; $P \leq 0.05$), and was found to worsen passing from the normal condition to the most severe (N: 12.7 vs WS-MOD: 11.0 vs WS-SEV: 7.92%; $P \leq 0.05$) (Petracci et al., 2013a). It can be inferred from the literature (Mudalal et al., 2015; Petracci et al., 2013a, 2015), that the meat ability to absorb brine solution is even lower in those fillets insulted by Wooden Breast and WSWB when compared to the unaffected and the WS conditions (on average, N: 14.7% vs WS: 9.40%, WB: 6.94% and WB/WS: 7.80%; $P \leq 0.05$). Besides a reduced ability to pick up marinade solution, severely WS and WSWB insulted breasts in particular exhibited a poor aptitude to retain it during refrigerated storage and cooking, as respectively indicated by higher purge (on average, N: 1.56; WS_SEV: 2.11; WSWB: 1.10%; $P \leq 0.05$) and cook loss (on average, N: 14.9; WS_SEV: 15.9; WSWB: 18.7%; $P \leq 0.05$) values (Petracci et al., 2013a; Mudalal et al., 2015). An impaired water holding and binding capacity resulted in an inferior total marinade yield ($P \leq 0.05$) characterising severe White Striping, Wooden Breast and WSWB cooked marinated fillets (on average, N: 89.2; WS_SEV: 82.2; WB: 87.3; WSWB: 85.6%) (Petracci et al., 2013a; Mudalal et al., 2015). Concerning texture, the presence of Wooden Breast, in particular in combination with white striations, makes the fillets notably harder to shear (N: 1.25 vs WSWB: 1.63 kg; $P \leq 0.05$) after tumbling and cooking (Mudalal et al., 2015).

As breasts affected by Wooden Breast and severe White Striping are rejected by the consumers at purchase, poultry processors are enforced to downgrade this meat instead of supply it to the fresh retailing. To partly reduce economic losses, poultry processors are enforced to utilize insulted meat to manufacture comminuted products (such as patties, nuggets, sausages, etc.). Considering the proven detrimental effect exerted by these emerging abnormalities on meat WHC and texture, a study aimed to evaluate the most feasible meat batter formulations to be adopted by the poultry meat processing industry, which permit the inclusion of insulted meat limiting the quality impairment. According to Qin (2013), the maximal percentage of Wooden Breast meat that can be incorporated

in the formulation of meat products avoiding perceived quality defects depends on the comminution method, as different techniques bring different meat particles size and shape and thus variations to the quality of meat products. Globally, relatively low Wooden Breast meat amounts can be mixed with normal meat. More in detail, those comminuted methods producing finer particles (for example, adopted to produce sausages) tolerate the inclusion of WB meat as 15% of the lean meat without significantly worsening chicken sausages quality. Differently, coarser grinding methods enable higher addition percentages, and a WB inclusion level of 30% in the coarsely chopped- and ground chicken nuggets causes no significant quality changes (Qin, 2013).

White Striping and Wooden Breast aetiology: a state of the art

At present, the aetiology of White Striping and Wooden Breast is not fully understood, as well as the connection relating these myopathies, even though the similar histopathological traits exhibited by the two conditions seem to suggest a common causative basis. Based on the detection of a different set of inflammatory cells characterising WS and WB affected tissues (Kuttappan et al. 2013a; Sihvo et al. 2014), it was even argued that these myopathies represent a broad spectrum of disease, with white striation occurring at early stage of muscle degeneration and tissue hardness increasing at a later stage (Sihvo et al., 2014). This last hypothesis is also supported by the evaluation of the meat quality traits, which result to be much more impaired by the occurrence of Wooden Breast alone or in combination with WS, rather than by only White Striping (Mudalal et al., 2015).

1. Investigation on predisposing factors

Many authors highlighted the association between broiler traits (increased growth rate, superior slaughter weight and breast yield, and therefore heavier and thicker breasts) and the incidence of both White Striping (Kuttappan et al., 2012a; 2013a, b; Petracci et al., 2013a; Lorenzi et al., 2014) and Wooden Breast (Sihvo et al., 2014). In particular, as an increased bird final weight was indicated to play a key role in myopathies occurrence and severity (Bauermeister et al., 2009; Kuttappan et al., 2013a; Lorenzi et al., 2014), the role of those factors modifying slaughter weight (genotype, faster growth rate, age, sex and diet energy content) was evaluated.

In the literature, there is no consensus about an involvement of genetics in Wooden Breast and White Striping onset. Many studies suggested that chickens' genotype might promote the occurrence of these myopathies (Kuttappan et al., 2013a; Petracci et al., 2013a; Lorenzi et al., 2014; Sihvo et al., 2014). In this regard, the findings of Lorenzi et al. (2014) clearly indicated that a high-breast yield genotype (body weight, BW: 3.8-4.2 kg) possessed higher percentages of both moderate ($P = 0.035$) and severe ($P = 0.032$) White Striping than peers belonging to a medium-sized genotype (BW: 3.0-3.8 kg). On the other hand, Bailey et al. (2015), even admitting the complex nature of these myopathies, sustain a strong non-genetic determinism for White Striping and Wooden Breast. According to these authors, environmental and management factors mostly contribute to the variance observed for White Striping (> 65%) and for Wooden Breast (> 90%); indeed, they obtained low correlation between these myopathies and body and breast weights, as well as low heritability for both WB (< 0.10) and WS (< 0.34). Also Russo et al. (2015) excluded a possible genetic predisposition to White Striping, as they found that WS and genetics were not correlated. Nevertheless, these last authors indicated that body weight and average daily gain are two significant predisposing factors, giv-

en the significant ($P < 0.01$) and strong correlations found between WS and body weight (0.69) and between WS and average daily gain (0.67).

As older birds reach heavier body weight, the age of chickens might play a significant role in myopathies occurrence and severity, as suggested by Lorenzi et al. (2014) and Kindlein (unpublished data). Comparing two groups of Cobb 500 chickens slaughtered at two different ages (35 and 42 d) and thus having different final body weight (2.62 and 3.37 kg, respectively), it clearly emerged that older (and heavier) birds had dramatic high percentages of total WS (71.0% vs 94.3%), WB (32.1% vs 89.5%) and WSWB (24.4% vs 84.7%) respect to younger birds, along with higher incidence of the severe White Striping degree (22.6% vs 65.1%). Even though it was observed that age exacerbates myopathic damages, there is no information concerning the age of onset of these myopathies to date.

As males have better performances than females in terms of live weight, weight gain, feed intake and feed conversion ($P < 0.001$), sex is expected to influence myopathies occurrence. These expectations were confirmed for Wooden Breast, as male broiler chickens exhibited higher WB incidences than females (M: 16.3% vs F: 8.0%; $P < 0.05$) (Trocino et al., 2015), but for White Striping, different scenarios were described. Indeed, Trocino et al. (2015) detected no discrepancies between the total, moderate and severe WS percentages between the two sexes. The findings of Lorenzi et al. (2014) were partly different: male chickens belonging to a medium-size genotype reported greater levels of moderate White Striping than the medium-sized females counterparts (M: 31.1% vs F: 21.1%; $P \leq 0.05$), but the percentage of severely affected muscles was similar for the two sexes (2.7%).

As feeding strategies can influence broiler chickens growth rate and weights, trials were conducted to compare the occurrence and severity of White Striping and Wooden Breast lesions in broiler chickens belonging to the same genotype but fed with different feeding strategies, such as low-energy vs high energy diets. As expected, a flock fed with two diets having different energy contents (low vs high) exhibited different outputs, as chickens fed with a reduced energy level (LED) reached inferior live and fillet weights compared to the high-energy (HED) counterpart (Kuttappan et al., 2012a). At the same time, however, better live performances exhibited by HED were accompanied by a lower percentage of breasts scored as normal (LED: 47.5% vs HED: 25.4%; $P < 0.05$) and a higher percentage of severely WS affected fillets (LED: 1.46% vs HED: 8.70%; $P < 0.05$). Consequently, chickens fed with a more energetic diet possessed enhanced performances than low- energy diets did, but at the same time, they exhibited an increased incidence of White Striping severity. Interestingly, the percentage of moderately white-striped breasts was statistically similar between HED and LED (LED: 51.1% and HED: 65.9%), as well as the total White Striping incidence (LED: 52.5% and HED: 74.65%). Therefore, on one hand, it could be postulated from these findings that

diets with a lowered energy content reduce White Striping severity; on the other hand, LED seems not to have a protective effect against White Striping occurrence in modern broilers (Kuttappan et al., 2012a).

Despite the histological similarities noticed between the two emerging myopathies and the nutritional muscular dystrophy, White Striping and Wooden Breast do not seem to be related to nutritional deficiencies, in particular, to a possible deficiency of vitamin E (Kuttappan et al., 2012b; Sihvo et al., 2014). This finding is supported by the evidence that increased vitamin E levels did not exert any significant preventing effect on WS occurrence and severity, as also chickens fed with adequate inclusion levels exhibited high degrees of White Striping (Kuttappan et al., 2015). Maybe, the lack of expected positive feedback from vitamin E supplementation is given by other concomitant conditions, which reduce or prevent the proper vitamin E supply from reaching breast muscle, such as a decreased capillary density (Kuttappan et al., 2012b).

Not all environmental and management potential causes of lesions have been explored to date. The relationship between emergent myopathies and coccidiostats has not been evaluated, even though ionophores were proven to exert a toxic action causing muscle tissue degeneration and necrosis in poultry (Sandercock & Mitchell, 2003; Chapman et al., 2010; Markiewicz et al., 2014) and in other species (Novilla, 1992).

2. Pathological mechanisms hypothesized

Before White Striping and Wooden Breast myopathies were discovered, the onset of other muscle pathological changes, which were also associated to the maximised chickens growth rate and yields, was observed during the last decades, and referred as growth-induced myopathies (Mahon, 1999; Sandercock et al., 2009; Petracci et al., 2015). One possible mechanism proposed to explain those myopathic damages involves the accidental selection for inadequate capillary growth and vascular supply accompanying the selection for increased muscular fibres size (Mahon, 1999). Indeed, pectoral muscle belonging to rapid growth hybrids is characterised by hypertrophy and reduced endomysium and perimysium spacing, as well as by an impaired capillaries/fibres ratio and augmented intercapillary distance at the same time (Wilson et al., 1990; Mahon, 1999; Velleman et al., 2003). This scenario adversely affects tissue metabolism, as cells receive reduced oxygen and nutrients supply (Hoving-Bolink et al., 2000), metabolic waste accumulate and muscular fibres become more vulnerable to oxidative stress (MacRae et al., 2006). Hereafter, the following increased ROS production within muscle fibres exerts a cytotoxic effect on proteins and membrane lipids, thereby threatening the cellular integrity (Powers et al., 2010); moreover, Ca^{2+} myofibril sensitivity and Ca^{2+} release from the sarcoplasmic reticulum could increase thus resulting in an impaired contractile activi-

ty (Allen et al., 2008).

According to the first studies conducted on White Striping and Wooden Breast, it seem that these two emerging myopathies share similar pathological mechanisms with the other skeletal muscle damages observed in modern broiler chickens. Firstly, oxidative stress might be listed among the contributors to Wooden Breast (Mutryn et al., 2015) and White Striping (Kuttappan et al., 2013a), as genetic selection for heavier breasts probably arose the vulnerability of white muscles to oxidative stress (Guetchom et al., 2012; Kuttappan et al., 2012). It was supposed that "white" glycolytic fibres of modern broiler chickens could be more sensitive to these conditions. Indeed, the occurrence of White Striping is more pronounced in "white" muscles such as those of breast (*Pectoralis major)* and thighs (*Iliotibialis*), but less visible on the surface of tenders (*Pectoralis minor*) and drumsticks (*Gastrocnemius*) muscles (Kuttappan et al., 2013a). Similarly, Wooden Breast condition has been observed in *Pectoralis major*, but it has not been detected in other skeletal muscles until now (Sihvo et al., 2014).

Breast thickness was suggested as another of White Striping and Wooden Breast predisposing factors (Kuttappan et al., 2013a), as it was noticed that both WS and WB mainly affect the cranial portion of the ventral surface (Kuttappan et al., 2013a), where the fillets achieve maximum thickness and convexity (Kuttappan et al., 2013a). Brewer et al. (2012) found a high correlation ($r = 0.81$) between fillet weight and cranial thickness; probably, enhanced growth rate could have result in a more accentuated hypertrophy toward the cranial area compared to the middle and to the caudal portions, thus leading to fibres overstretching, impaired blood supply (ischemia), tissue damage and initiation of reparative responses in (Kuttappan et al., 2013a). Increased performances and tissue ischemia are common predisposing factors of WS and WB, as well as of deep pectoral myopathy (DPM) (Bailey et al, 2015; Petracci et al., 2015), as revealed by the similar histological lesions detected in WS and WB *Pectoralis major* and those of DPM affected *Pectoralis minor*.

Another possible pathological mechanism involves a possible connection existing between the two myopathies and an unbalanced cells cations regulation, as increased Na^+, K^+, Mg^{2+} and Ca^{2+} levels were detected in correspondence to previously described muscular tissue damages affecting high growth rate broiler chickens (Sandercock et al., 2009). In detail, uncontrolled and prolonged overload of calcium within the cell (from extracellular sources, or mobilized from the sarcoplasmic reticulum) was demonstrated to be a triggering factor for muscle tissue degeneration (Jones et al., 1984; Duncan & Jackson, 1987; Jackson, 1993; Sandercock & Mitchell, 2003, 2004). As calcium is an activator of proteases and lipases, such as phospholipase A_2 (Jackson et al., 1984; Jackson, 1993; Sandercock & Mitchell, 2003), one possible mechanism could be the alteration of the cell membrane integrity and the following loss of cell functionality mediated by Ca^{2+}- activated enzymes (Mitchell,

1999; Sandercock & Mitchell, 2003). An excessive intracellular calcium accumulation could also affect the myofibers hypercontraction, which could ultimately result in the cells "hyaline" degeneration and necrosis (Tay et al., 1992). An excessive calcium release from the sarcoplasmic reticulum, resulting in enzymes activation and protein denaturation (Sandercock & Mitchell, 2003), could also be a triggering mechanism of the pale, soft and exudative (PSE) condition (Strasburg & Chiang, 2009), which was reported also in broiler chickens (Zhang & Barbut, 2005). Even though White Striping and Wooden Breast do not exhibit gross similarities with PSE, this observation contributes in proving the role of excessive calcium build-up in muscular lesions onset. Interestingly, Sandercock & Mitchell (2004) speculated that calcium uptake into muscle cells might be mediated by an increased intracellular Na^+ level in chicken selected for broiler traits, which was observed by several authors (Sandercock et al., 2009), through the involvement of the Na^+/Ca^{2+} exchanger (Sandercock & Mitchell, 2004). Therefore, muscle injury in rapid growth birds seems also to be associated with a disturbed sodium homeostasis; according to Fuller et al. (1976), also a moderate phosphorus depletion could lead to muscle cell damage, as a lowered level of this element results in a reduce synthesis of adenosine triphosphate (ATP). The consequent lack of energy may interfere in the activity of the Na^+/K^+- ATPase in maintaining a high concentration of potassium ions within the cell; as a result, abnormally increased intracellular Na^+ and Cl^- ions, along with the decline of normal K^+ concentration and transmembrane potential follow. In addition, as less ATP molecules are available for sarcolemma metabolic processes, also a greater myofiber permeability to sodium compromising sarcolemma integrity can be speculated (Fuller et al., 1976).

The selection for broiler traits accounted for the onset of several previously described conditions, such as inherited muscular dystrophy, thermal stress, trauma, myopathies with exertional, nutritional or toxic origin affecting *Pectoralis major* muscle (Klasing et al., 2008), as well as the deep pectoral myopathy of *Pectoralis minor* (Bianchi et al., 2006) and the myodegeneration of the *anterior latissimus dorsi* (ALD) muscle (Kuttappan et al., 2012b; Zimermann et al., 2012). Both Wooden Breast and White Striping were found to possess microscopic similarities with these conditions; in particular, degeneration, necrosis, and regeneration with fibrosis also were found to characterize ALD myopathy (Sihvo et al., 2014) as well as tissue damage following nutritional deficiencies of selenium and vitamin E in the diet (Guetchom et al., 2012). Macroscopically, the detection of white striations on broiler breast fillets was described by Valentine and McGavin (2012) as the gross manifestation of mineralization, or collagen or fat infiltration potentially due to any cause. Previously, this phenomenon had already been observed as clinical gross feature of nutritional myopathy due to inadequate vitamin E and associated nutrients level (Klasing et al., 2008), or occurring in hereditary muscular dystrophy (Asmundson & Julian, 1956; Julian & Asmundson, 1963). As for Wooden Breast,

some macroscopic similarities were detected with ALD myopathy, such as paleness, increased muscle thickness and exudate covering the surface; nevertheless, the palpable hard consistency of the pectoral muscle remains a prerogative of WB (Sihvo et al., 2014).

It emerged that novel myopathies were not associated to infection or inflammation, as infectious agents such as bacteria or parasites were not detected (Kuttappan *et al*., 2013b; Sihvo *et al*., 2014). The lack of any difference in the various hematologic parameters between WS unaffected and affected chickens suggests a normal liver function also in the latter group (Kuttappan et al., 2013b). Similarly, kidneys also were found to maintain their normal functions: the normal nitrogen, creatinine, phosphorus, and uric acid levels detected in the blood of WS insulted birds (Hochleithner, 1994), as well as the normal serum levels of total protein, albumin and glucose, indicate a proper absorption or metabolism of these compounds (Kuttappan et al., 2013b). Therefore, it was demonstrated that these emerging myopathies do not possess a systemic character; conversely, they are specifically associated with tissue damage, as suggested by the increased levels of creatine kinase, alanine transaminase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) in the serum, probably due to their leakage from those fibres undergoing degeneration (Kuttappan et al., 2013b).

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CHAPTER 2

Objectives and outline

The studies conducted so far underlined the association between the occurrence and severity of WS and WB myopathies and the continuous challenging birds to reach ever higher weights and yields in ever shorter times. It is clear that these conditions have a complex and multifactorial origin; however, the precise causative basis and all the pathological mechanisms behind myopathic changes onset remain unclear to date, as well as the knowledge about the predisposing factors and lesions morphology is not complete. Because of the impaired visual appearance of affected breasts, poultry industry is enforced to downgrade fillets and to use them for processed products. Up to date, poor information is available on the consequences of WS and WB on meat technological and sensory traits, even though the first studies indicated a negative impact on meat quality. For all the above-mentioned considerations, this PhD thesis investigated aspects not yet considered by other researchers, having as ultimate goal that of offering to scientific community and poultry processors knowledge hopefully useful to reduce the economic impact of these defects.

As the knowledge regarding WB pathological changes is still scarce, Chapter 3 focuses on typing the morphology of macroscopic and histologic lesions. Firstly, the incidences of the characterizing WB macroscopic "descriptors" were calculated. At histological level, previous literature indicates that the occurrence of giant cells is a phenomenon linked to myopathy and poor meat quality. Therefore, researching a possible difference in giant cells incidence between WB and normal breast was thought to be of interest. Clearly, due to their impaired visual appearance, WS and WB meat cannot be sold fresh but it has to be downgraded to manufacture processed products. However, tissue myodegeneration occurring in WS and WB at histologic level could impair those quality traits that are fundamental for meat processing. To evaluate this hypothesis, the technological quality traits of WB fillets (pH, colour traits, WHC and shear force) were tested in Chapter 3, while the same parameters were tested for WS and WSWB fillets in Chapter 4. As sensory traits of cooked affected meat had never been outlined before, Chapter 4 provides the sensory profiles of fillets affected by WS and WSWB along with meat mineral profile, which was analysed in the same experiment to support the hypothesis that a defective cation regulation is present in WS and WB myopathies.

To date, not all potential causes of WS have been fully investigated; among them, the role of some farming practices in myopathies breaking out has to be clarified. For example, no studies have explored the link between anticoccidials and WS, even though the toxicity of coccidiostats in skeletal muscle has already been reported in broiler chickens and other species. The study in Chap-

ter 5 evaluated WS occurrence and severity in breasts of chickens subjected to two coccidiosis control programs (vaccine vs anticoccidial additive) and fed diets with different energy density and bioavailability of nutrients. Moreover, the observation of the breasts of chickens at different ages (12, 25 and 51 d) was intended to identify the age of onset of WS.

WB is primarily characterized by a hard palpatory consistency of affected breasts, which has been ascribed to fibrosis so far. However, some cases of hard breasts without a significant collagen accumulation were also detected; this phenomenon opened new hypotheses on the reasons for WB hardness development. Starting from the association between muscle microstructure and water molecules distribution within the muscle, the study described in Chapter 6 tested the role of myowater to explain the typical WB hardness, integrating nuclear magnetic resonance (NMR) measurements with texture analysis. NMR properties and hardness were evaluated according to not only muscle condition but also considering a 72 h *post mortem* chilled storage and four different sampling locations within the breast, as these factors have been shown to affect meat textural properties.

CHAPTER 3

Effect of "Wooden Breast" Appearance on Poultry Meat Quality, Histological Traits, and Lesions Characterization

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ABSTRACT

The purposes of the study were to investigate the effects of Wooden Breast (WB) myodegeneration on poultry meat quality and to give a contribution in typing lesions morphology. At a poultry meat cutting facility, 474 carcasses of a high-breast-yield hybrid chickens were inspected for WB condition, and 30 normal (N) and 30 affected (WB) breast fillets (*Pectoralis major*) were randomly selected. The WB condition represented 53.2% of the examined carcasses. Weight, cross sectional area (CSA), pH, L*, a*, b* colour values, water-holding capacity, and Warner-Bratzler shear force were determined. Samples were also visually and histologically evaluated. Affected samples were heavier, thicker, paler ($P < 0.001$), and characterized by palpatory hardness and lower water holding capacity ($P < 0.05$). Macroscopically, abnormalities were primarily localized in the cranial portion of the fillet and defined by the presence of bulges, petechiae, fluid and clear exudate, and higher pH. Microscopically, the WB condition was characterized by muscle fibres with greater CSA ($P < 0.001$) and higher giant fibres prevalence ($P < 0.01$). Data suggest a relationship between breast weight and WB condition.

Keywords: emergent myopathy; breast muscle defect; myodegeneration

1. Introduction

An ever-increasing demand for chicken meat has enforced poultry supply chain to improve growth rate and breast meat yield of broilers, but this trend led to a substantial onset of breast muscle defects at the same time (Dalle Zotte et al. 2015, Chapter 5). An emergent myopathy has been described and named Wooden Breast (WB) (Sihvo et al. 2013). Distinguishing macroscopical traits of the affected *Pectoralis major* muscle are the remarkable palpatory hardness and the pale colour, as well as the presence of bulges, small haemorrhages, and a slimy surface due to a fluid and clear exudate covering the hardened areas. Interestingly, also the observation of White Striping (WS) and extended areas with separated muscle bundles is often concomitant. Histologically, WB condition is a moderate or severe myodegeneration, accompanied by necrosis, inflammatory cells accumulation, and reparative responses (fibrosis). Abnormalities in cells structure and shape (internalization of nuclei, split cells appearance, and loss of poligonality) are other typical traits of tissue damage.

Although the underlying causes of the disease remain obscure, through the analysis of genes expression, Mutryn et al. (2015) indicated that hypoxia, oxidative stress, increased intracellular calcium as well as the presence of fibre-type switching could be reasonably key features of WB. In addition, this myopathy demonstrates similar histological traits as WS, whose aetiology has just been explored even though not completely. Fast growth rate and ever-increasing breast meat yield reached by birds seemed to be responsible for breast muscle overstretching and capillary density lowering, with ischemia, inflammation, and reparative fibrotic response as consequence (Kuttappan et al. 2013). Selection for increased muscle size, together with muscle type, slaughter age, and energy level of the diet, is also a key factor linked to higher giant fibres (GF) incidence, phenomenon observed in turkey (Remignon et al. 2000), rabbit (Dalle Zotte et al. 2001), chicken (Miraglia et al. 2006), and swine (Schubert-Schoppmeyer et al. 2008). These cells were described as oval or rounded-shaped, with larger cross-sectional area, distributed in groups or isolated at the periphery of fascicles (Sink et al. 1986; Remignon et al. 2000) and characterized by altered energy metabolism and contraction speed (Dalle Zotte et al. 2001). In addition, according to Dalle Zotte et al. (2001), GF are associated with modification of the fibre type proportion of the muscle. In fact, despite being normally composed by white fibres (αW), rabbit *Biceps femoris* muscle presenting giant fibres was found to exhibit in- creased percentages and area of red fibres (βR), which were positively correlated to higher ultimate pH values. Giant cells were thought to arise from hypercontracted individual fibres (Dutson et al. 1978; Sink et al. 1986; Sosnicki 1987), to be a sign of myopathy (Sosnicki 1987) and fibres being in a degenerative, pre-necrotic stage (Cullen et al. 1979; Wegner and Ender 1990; Wilson et al. 1990). In any case, GF were correlated with poor meat qual-

ity in pigs (Essen-Gustavsson 1995; Fiedler et al. 1999, 2004). On the basis that the outbreak of GF seemed to be the faster growth (Miraglia et al. 2006) and the selection for increased muscle size (Sosnicki et al. 1991), researching a possible difference in the prevalence of giant fibres in WB affected and normal meat samples could be of interest. Poor information is also available about WB consequence on meat technological traits, whereas the detrimental implication of WS on these variables has already been explored. Therefore, the present study tried to gain additional information on the quality impairment of WB affected breast, and then it tried to deepen the knowledge of macroscopic typifying descriptors and of the histological traits.

2. Material and Methods

At a poultry-meat cutting facility, 474 carcasses of a high-breast-yield hybrid chickens (unknown gender) were 48 h post mortem subjected to visual and tactile inspection for WB condition. Subsequently, 30 normal (N) and 30 WB affected breast fillets were randomly selected. WB condition was observed in 252 carcasses, which represented 53.2% of the examined carcasses. Affected samples were visually examined for the WB descriptors, according to Sihvo et al. (2014), by filling up a dedicated form presented elsewhere (Tasoniero et al. 2016, Chapter 4). Selected right breasts were weighed, then on their cranial and caudal ends, pH was measured in duplicate by infission with a portable pH-meter FE20 (Mettler Toledo, Switzerland), as well as colour values of lightness, redness, yellowness, chroma, and hue (L*, a*, b*, C*, and H°, respectively) with a RM200QC colorimeter (X-Rite Co., Germany). Samples were individually vacuum-packed and kept frozen for 3 months at –20 °C; thereafter, fillets were thawed to determine the thawing losses, again vacuum-packed, and cooked in a water bath until core temperature of 74 °C to determine the cooking losses.

Shear force was assessed with a TA-HDi Texture Analyzer (Stable Macro System, UK) on 6 cylinder- shaped cooked meat pieces per breast (Ø 1.25 cm). Samples were obtained through the whole breast thickness; therefore, surface and deep layers were both included. Then, a Warner-Bratzler cell (100- kg load cell, 2 mm/s crosshead speed) inserted in the texturometer cut samples perpendicularly to the fibres direction. The Warner-Bratzler shear force (WBSF) values of each sample represented an average of the 6 measurements.

Cross sectional area (CSA) of the fillet and histological observations were performed on the left half- breasts. CSA data were obtained cutting transversely the cranial portions and photographing the views with Canon EOS 500D camera with 18–200 mm/3.5–5.6 lens; images were subsequently processed with digital image software (Carl Zeiss, Model Axiovision 4.6.3.0). Breast samples (15 N and 15 WB) were taken from the central-external part sampling both the surface and the deep

layers of *Pectoralis major*, fixed in 10% formalin, processed by conventional methods and embedded in paraffin wax. Then, samples were cut at a 4-µm thickness for hematoxylin-eosin (HE) staining (to estimate average normal fibres and giant fibres number and size) and for Masson's trichrome staining (to measure collagen and empty spaces percentages). Computerized image analysis (Buche 1990) was used to determine the fibre mean CSA (of both normal and giant fibres), the collagen and empty space area, and to count the average frequency of giant fibres per unit size of muscle section. Breast quality traits, CSA, weight and histological data were analyzed through one-way ANOVA of the SAS software (Statistical Analysis System, Version 9.3, 2004), considering treatment (N vs WB) as independent variable. Descriptors were examined by $\chi 2$ and z tests.

3. Results and Discussion

Wooden Breast-affected fillets were heavier (505 vs 377 g, respectively; $P < 0.001$) and exhibited greater CSA than N fillets (30.3 vs 25.1 cm^2, respectively; $P < 0.001$) (Figures 3.1 and 3.2); a high correlation ($r = 0.81$) between the two parameters was found (Brewer et al. 2012).

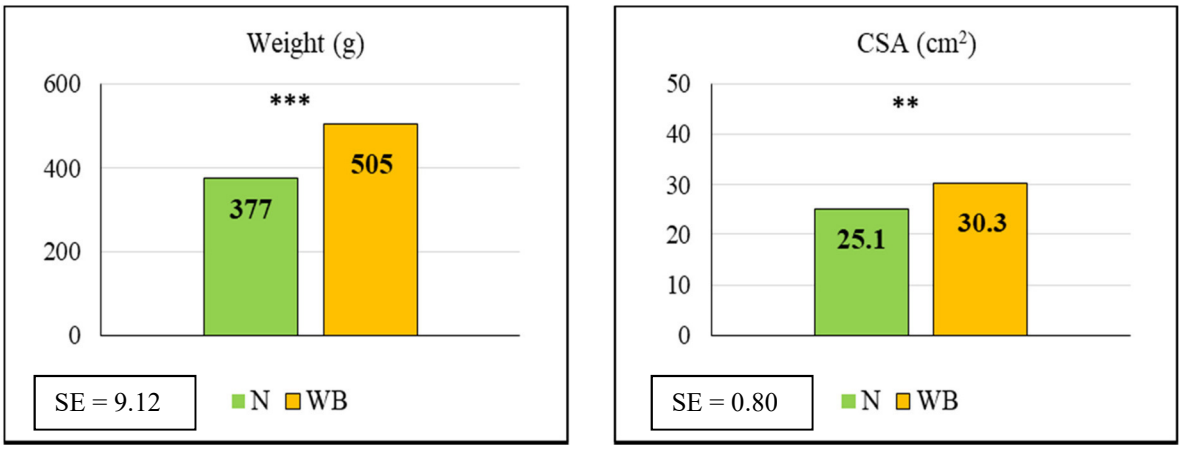

Figure 3.1 Breast weight of normal (N) and Wooden Breast (WB) affected birds; ***P < 0.001.

Figure 3.2. Breast cross sectional area (CSA) of normal (N) and Wooden Breast (WB) affected birds **P < 0.01.

At 48 h post mortem, WB samples were characterized by significantly higher pH and colour values and these differences were maintained after the frozen storage (Tables 3.1 and 3.2). As demonstrated by higher pH and higher a* and b* values, breast cranial portions suffered more from the WB condition ($P < 0.01$); probably similarly to WS, the maximum thickness observed toward the cranial ends is responsible for over-stretching and ischemia, which resulted in tissue damage and reparative responses (Kuttappan et al. 2013). The higher pH values observed in WB breasts than in N ones may be explained by the strong negative correlation between glycogen storage and breast muscle weight (Le Bihan-Duval et al. 2008). Therefore, a breast with greater size could exhibit a reduced glycolytic potential, resulting in higher ultimate pH. In fact, it was recently hypothesized that the high pH of WB fillets could be related to an altered glycogen utilization, which would result in glycogen depletion (Soglia et al. 2016a). Differently, breast redder portions could be related to a higher myoglobin content: the fibre-type switching phenomenon, which was found to occur in WB condition, seemed to lead to a higher expression of myoglobin genes (Mutryn et al. 2015). Our results concerning yellowness index are in accordance with previous results on WS (Petracci et al. 2013) and WB (Mudalal et al. 2015): affected fillets are more yellow than non-affected ones, probably due to the severe fibrotic response. On the contrary, both the portions (cranial and caudal) were brighter in affected samples ($P < 0.001$), thus confirming the evidence of the importance of this

last feature in the WB condition. Chroma (C*) and hue (H°) followed the similar trend of redness value.

Table 3.1 Breast (*Pectoralis major*) pH and L*, a*, b* colour values at 48 h *post mortem.*

	N[1]	WB[2]	Significance	RSD[3]
pH cranial	5.90	6.03	**	0.19
pH caudal	5.87	5.92	ns	0.16
L* cranial	50.9	54.6	***	3.4
L* caudal	50.5	53.8	***	3.3
a* cranial	–1.1	–0.3	**	1.4
a* caudal	–1.2	–0.6	ns	1.4
b* cranial	12.9	15.9	**	3.1
b* caudal	12.4	13.3	ns	3.6
C* cranial	13.0	16.0	**	3.2
C* caudal	12.6	13.4	ns	3.6
H° cranial	95.4	90.5	**	5.2
H° caudal	96.8	94.3	ns	7.3

Table 3.2 Breast (*Pectoralis major*) pH and L*a*b* colour values after frozen storage.

	N	WB	Significance	RSD
pH cranial	5.91	6.03	***	0.12
pH caudal	5.86	5.91	ns	0.13
L* cranial	48.5	52.3	***	3.0
L* caudal	48.9	51.3	***	2.4
a* cranial	–0.4	–0.7	**	1.3
a* caudal	–1.2	–1.2	ns	1.3
b* cranial	16.7	19.8	**	3.7
b* caudal	13.9	15.2	ns	3.7
C* cranial	16.7	19.9	**	3.7
C* caudal	14.1	15.4	ns	3.6
H° cranial	92.1	88.8	**	4.6
H° caudal	96.0	96.0	ns	6.2

L*: lightness, a*: redness, b*: yellowness, C*: chroma, H°: hue, N: normal, WB: Wooden Breast, RSD: residual standard deviation, ns: not significant, $^{**}P < 0.01$, $^{***}P < 0.001$.

Our WB fillets also displayed a worse water holding capacity (WHC), as demonstrated by the superior cooking losses ($P < 0.01$), which contributed to the higher total losses ($P < 0.05$) observed for this group (Table 3.3). Such result is in accordance with recent literature data (Tijare et al. 2016). Muscles could exhibit a reduced ability to hold water because of the myodegeneration. It is likely that, as previously observed in WS condition (Petracci et al. 2014), the functional proteins content, responsible for meat WHC is lowered in favour of collagen (Dalle Zotte et al. 2014; Petracci et al.

2015). This hypothesis found confirmation in the work by Soglia et al. (2016a), which showed a higher collagen and lower protein contents in chicken breast muscles affected by WB abnormality compared to normal ones. This replacement results in a significant impairment of protein functionality and water retention of cooked meat, despite collagen proteins shorten, swell, solubilize during the cooking process and gelatinize when cooled (Palka 1999; Tornberg 2005), thus keeping an amount of water. Furthermore, WB myopathy was found to worsen the oxidative status of meat proteins and lipids, which could contribute to reduced storage stability of meat and thus WHC (Soglia et al. 2016b). Despite the results of a recent work by Soglia et al. (2016a) showed that WB chicken breasts exhibited higher hardness, gumminess, and chewiness than normal ones, shear force values of the affected cooked breasts of the present experiment did not correspond to the palpatory hardness perceived at the fresh and thawed state; these results are in accordance with those of Mudalal et al. (2015). The extensive poor cohesion and the tendency of the fibre bundles to separate, mainly at the cranial level, could have mitigated the expected hardness (Petracci and Cavani 2012; Petracci et al. 2013). In addition, toughness values could have been mitigated also because of the storage period: the large extracellular ice crystals, formed during freezing, could have broken down myofibrils apart, thus resulting in physical structure disruption (Leygonie et al. 2012) (Table 3.3).

Table 3.3 Breast (*Pectoralis major*) weight, water holding capacity (WHC), and Warner-Bratzler shear force (WBSF).

	N	WB	Significance	RSD
Thawing losses (%)	5.4	5.0	ns	1.7
Cooking losses (%)	23.5	26.4	**	3.7
Total losses (%)	29.0	31.4	*	4.4
WBSF (N)	15.5	16.9	ns	2.8

N: normal, WB: Wooden Breast, RSD: residual standard deviation, ns: not significant, $^{**}P < 0.01$, $^{***}P < 0.001$.

Macroscopically, the descriptors used highlighted that lesions are distributed mainly in the cranial end of the fillets ($P < 0.001$) (Table 3.4). The presence of bulgies (in 100% of the cases) was one of the selection traits, and it was localized at the cranial level in 60% of the observations, as well as the hard palpatory consistency and the colour ranging from normal to pale. The exudate appeared with a prevalence of 43.4% and it was mainly fluid and clear ($P < 0.01$), whereas pinpoint haemorrhages were present in 30% of the cases and mostly in the upper area. On WB samples the WS condition was present in 90% of the cases, thus confirming the evidence that such myopathies often occur simultaneously (Tijare et al. 2016), and it was mainly located in the cranial side of the breast, where the muscle displayed maximum thickness.

Table 3.4 Wooden Breast (WB) descriptors prevalence.

Descriptors				Significance
Localization, %				
Cranial	**Caudal**	**Cra + Cau**	**Longitudinal**	
56.7^{A}	3.3^{B}	23.3^{B}	16.7^{B}	***
Breast colour, %				
Normal	**Pale**	**Pink**	**Other**	
36.7^{A}	56.7^{A}	3.3^{B}	3.3^{B}	***
Breast consistency, %				
Hard	**Very hard**			
80.0^{A}	20.0^{B}			***
Bulgies localization, %				
Cranial	**Caudal**	**Cra + Cau**	**Longitudinal**	
60.0^{A}	0.0^{C}	23.3^{B}	16.7^{BC}	***
Exudate consistency, %				
Fluid	**Turbid**			
36.7^{A}	6.7^{B}			**
Exudate colour, %				
Clear	**Grey**	**Yellow**		
40.0^{A}	0.0^{B}	3.3^{B}		***
Hemorrages localization, %				
Cranial	**Caudal**	**Longitudinal**		
23.3^{A}	0.0^{B}	6.7^{AB}		**
Hemorrages width, %				
Pinpoint	**3-5 mm**	**≥5 mm**		
23.3^{A}	6.7^{AB}	0.0^{B}		**

**: P<0.01; ***: P<0.001

At histological level, fibres of WB samples were identified for their larger CSA ($P < 0.001$) and for the higher incidence of giant-type fibres among them ($P < 0.01$) (Table 3.5). This lowered the fibres average number visualized in the field area (80 vs 105; $P < 0.001$). It is worthy to be noticed that giant-type fibres belonging to WB samples possessed CSA values that were far greater than those of the giant-type fibres of the non-affected counterparts (6070 vs 3816 μm^2; $P < 0.01$). The occurrence of giant-type fibres and of fibres with an increased size are indexes of abnormality in the muscle architecture and are typical of the degenerative process which establishes itself in breast muscle of fast growing and higher yield birds (Petracci et al. 2013). A microscopic analysis revealed that they are hyper-contracted fibres with a sign of structural disintegration; in these cells, organelles important for cell integrity and functionality are damaged, resulting in an impaired adenosine triphosphate production (Schubert-Schoppmeyer et al. 2008). Contrary to the expectations, but likely due to the limited sampling size, in the present study the pathological condition did not entail an enhancement concerning collagen percentages, and only numerically higher values were observed. Nevertheless, it remains undeniable that collagen proliferation is a typifying characteristic (Sihvo et al. 2013).

Table 3.5 Histological traits.

	N	WB	Significance	RSD
Average fibres number (on total field area)	105	80	***	14.7
Fibres CSA (% total field area) (HE staining)	71.0	73.5	ns	4.5
Fibre CSA (μm^2)	2012	2750	***	332
Giant fibre CSA (μm^2)	3816	6070	**	2111
Giant fibres (% total fibres number)	1.1	2.0	**	0.8
Giant fibres area (% total field area)	2.1	3.8	**	1.6
Collagen area (% total fibres area)	16.1	20.0	ns	7.2
Collagen area (% total field area)	10.7	12.9	ns	3.4
Empty spaces (% total field area)	21.4	19.0	ns	4.5

N: normal, WB: Wooden Breast, RSD: residual standard deviation, CSA: cross sectional area, HE: hematoxylin-eosin staining,
ns = not significant, ** $P < 0.01$, *** $P < 0.001$

4. Conclusion

Wooden Breast myodegeneration worsens meat quality traits and the visual appearance of the affected breasts through the presence of characterizing lesions. Despite the studies that have been conducted until now, many aspects on WB aetiology remain unclear. Considering the analogies with WS condition, high body weight and superior breast yield reached by birds within a short period of time could contribute to the myopathy. However, the pathological mechanisms at molecular level and the implication of the genetic component in the development of WB remain obscure.

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CHAPTER 4

Technological quality, mineral profile, and sensory attributes of broiler chicken breasts affected by White Striping and Wooden Breast myopathies

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ABSTRACT

The aim of the research was to study the impact of White Striping and Wooden Breast myopathies on the technological quality, mineral, and sensory profile of poultry meat. With this purpose, a total of 138 breasts were selected for a control group with normal breasts (N = 18), a group of breasts characterised by white striping (WS = 60) myopathy, and a group of breasts having both white striping and wooden breast myopathies (WSWB = 60). Data revealed that the simultaneous presence of the two myopathies, with respect to the WS lesion individually considered, had a further detrimental effect on pH (6.04 vs. 5.96; $P < 0.05$), yellowness (11.4 vs. 10.3; $P < 0.01$), cooking losses (30.4 vs. 27.6%; $P < 0.05$), toughness instrumental values (22.8 vs. 20.0 N; $P < 0.01$), and perception (6.22 vs. 5.56; $P < 0.01$). In addition, mineral contents suggest that a defective ions regulation is also present in white striping and wooden breast myopathies.

Key words: chicken breast, White Striping, Wooden Breast, physicochemical analyses, sensory profile.

1. Introduction

An ever-increasing demand for chicken meat has made the poultry industry focus on high-energy diets and intensive selection of genotypes exhibiting faster growth with higher breast yields. However, this trend happened at the same time as the onset of two emerging meat quality issues: White striping (WS) and wooden breast (WB). Macroscopically, WS appears as white striations running parallel to the muscle fibers (Bauermeister et al., 2009; Kuttappan et al., 2012a,b; Kuttappan et al., 2013a), which give a fatty, marbled, and abnormal appearance to the fillets (Kuttappan et al., 2012b). In addition, fiber bundles beneath the striated area can display a tendency towards separation (Petracci et al., 2013). On the other hand, WB condition is characterized by remarkable palpatory hardness and paleness, along with a fluid and clear exudate, bulges, small hemorrhages, and white striping, as well as areas with separated muscle bundles, particularly within the deep layers of the muscle (Dalle Zotte et al., 2014; Sihvo et al., 2014). Histologically, both WS and WB lesioned areas show myodegeneration along with variable amount of interstitial connective tissue accumulation (Kuttappan et al., 2011, 2013a; Sihvo et al., 2014). Moreover, WS is also characterized by lipidosis (Kuttappan et al., 2013a; 2011). The triggering factors of WS are: heavier birds (Bauermeister et al., 2009; Kuttappan et al., 2013b), increased growth rate (Kuttappan et al., 2012a; Mudalal et al., 2014), and breast yields (Lorenzi et al., 2014; Mudalal et al., 2014), as well as thicker fillets (Kuttappan et al., 2013b) have all a role in WS condition development. As for WB condition, the etiology is still obscure, however, similarly to WS, the greatest suspicions seems to fall on the fast growth rate and the ever-increasing breast meat yield (Dalle Zotte et al., 2014; Mudalal, et al., 2015). The onset of the two defects seems to be also influenced by the part considered: the maximum thickness observed in the cranial part could produce overstretching or ischemia resulting in tissue damage and reparative responses (Kuttappan et al., 2013a) due to an impaired blood supply (Hoving-Bolink et al., 2000). The muscle considered could also play a significant role, suggesting that red muscles are less sensitive than white muscles. Indeed, *Pectoralis major* and *Iliotibialis* exhibited higher WS incidence than *Gastrocnemius* (Kuttappan et al., 2013a); WB has been described in the breast fillet (Sihvo et al., 2014) and *Anterior latissimus dorsi* muscle has been reported to exhibit similar lesions (Zimermann et al., 2012). Genetic selection for broiler traits could also involve a defective cations regulation, especially of calcium, which seems to be associated to myopathic changes and meat quality problems (Sandercock et al., 2009). Interestingly, some farming practices could be of importance in myopathies breaking out. A recent study of Dalle Zotte, et al. (2015b) (Chapter 5) revealed that the use of ionophores against coccidiosis have a predisposing action towards WS; indirectly, due to their positive effect on intestinal integrity and live performances, and directly, due to their proven myotoxicity (No-

villa, 1992; Chapman et al., 2010; Markiewicz et al., 2014). The average frequencies observed so far under commercial and experimental conditions are higher than 50% both for WS (Kuttappan et al., 2012a; Petracci et al., 2013; Lorenzi et al., 2014; Dalle Zotte et al., 2015b, Chapter 5) and WB (Dalle Zotte et al., 2015a). The high percentages achieved, together with the negative impact on the various meat quality aspects, demonstrate how this myopathy is becoming an issue of great relevance for the poultry industry. Indeed, meat is less suitable for processing (Kuttappan et al., 2012a; Petracci et al., 2013; Petracci et al., 2014; Mudalal et al., 2015) enforcing producers to manufacture processed products instead of offering breasts for fresh retailing, and the visual acceptability at purchase is compromised (Kuttappan et al., 2012b; Petracci et al., 2013). Nevertheless, no information is currently available on WS and WB myopathies related to sensory analysis; attributes of affected breasts different from the visual appearance have not been tested so far. Therefore, the aim of the present study was to outline a sensory profile of affected cooked breasts, investigating a possible negative impact exerted by WS and WSWB beyond the visual appearance using a trained panel. Additional information on the technological quality traits of breast meat simultaneously affected by the two myopathies is provided; moreover, researchers chose to investigate the mineral profile to give a contribution in affirming that an altered cellular ions balance could be one of the triggering factors.

2. Materials and Methods

Sample Collection

For this study, breast muscles belonging to a batch of 500 carcasses of 51-day-old Ross 708 male chickens were used. Birds were processed in an authorized commercial slaughterhouse by electrical stunning (120 v, 200 Hz). Subsequently, they were soft-scalded (53 °C for 2 min) and air-chilled after evisceration (precooling at 5 °C for 60 min, followed by chilling at 0 °C for 90 min). Carcasses were then transported to a poultry cut-up plant and stored at 4 °C; there, breasts were deboned and sorted, then collected by the working team members forty-eight hours *post-mortem*. Sorting procedure consisted in a visual and palpatory inspection to detect the presence on breasts (m. *Pectoralis major*) of lesions attributable to WS (Kuttappan et al., 2013a) and WB (Sihvo et al., 2014). The WS selection criteria included the presence of visible white striations on the surface. A grid arranged according to the scheme proposed by Puolanne (personal communication) and recently used by Dalle Zotte et al. (2015a), helped the researchers to identify WB samples. WB detection considered first the presence of hard areas at palpation of both the left and right breast muscles, then, detection and characteristics of other descriptors (colour, bulges, exudate, haemorrhage, and concomitant presence of WS) were taken into account (Table 4.1). Initially, four experimental groups of 60 breasts each were planned: a

group with no macroscopic lesions (Normal group, N), a group exhibiting WS myopathy (WS), a group with WB myopathy (WB), and a fourth group having the both lesions (WSWB). As only eighteen N and no WB samples were found, only three experimental groups were formed and a total number of 138 whole breast muscles (18 N, 60 WS, and 60 WSWB) were collected and weighed.

Instrumental Analysis

At the Department of Animal Medicine, Production and Health (MAPS) laboratory, *Pectoralis major* muscles were subjected to instrumental analyses. A total of 138 right breasts (18 of the N group; 60 belonging to WS; and 60 of the WSWB group) were used to measure pH and L* a* b* color values. On 108 of them (8 Normal, 50 WS, and 50 WSWB) the cooking losses and the Warner-Bratzler shear force (WBSF) were determined. A total of 113 left breasts (10 Normal, 52 WS, 51 WSWB) were used for thawing and cooking losses determination after a six-month storage. The remaining 24 left *Pectoralis major* muscles (8 samples per treatment) were considered for the mineral profile analysis. On the cranial and caudal ends, ultimate pH was measured with Mettler Toledo FE20 and colour values (CIE 1976 L* a* b*) were detected with RM200QC (X-Rite, Co, Neu-Isenburg, Germany) colorimeter provided with multidirectional LED illumination set at D65 10◦. The colour measurements were taken with the instrument resting on samples kept in the horizontal plane. Fillets were then individually vacuum-packed in polypropylene bags, put in a water bath set at 80 °C, and cooked until a core temperature of 78 °C was reached. At each cooking round, the fillets were divided into two main groups according to weight, and core temperature was controlled by inserting the probe of two digital thermometers (Checktemp 1 Digital Thermometer, Mod. HI98509, Hanna Instrument, Limena, Padova, Italy) in the thickest part of the heaviest and of the lightest fillet. Once cooked, samples were cooled by immersing bags in cold water added with crushed ice until room temperature was reached; then bags were removed and breasts were reweighed to determine the cooking losses as a percentage of the initial sample weight.

WBSF was assessed on cylindrical cooked meat pieces (1.25-cm diameter × 2 cm length) obtained by coring the muscle with a mechanical coring device (Perlo et al., 2010) oriented parallel to the fibres direction (Wheeler et al., 1996). Six cylindrical samples (3 from the cranial portion and 3 from the medial caudal portion) were attained from each breast, by cutting the muscle through the whole breast thickness; therefore, surface and deep layers were both included. Thereafter, cylinders were cut perpendicularly to the muscle fibre direction with a Warner-Bratzler cell (100 kg load cell, 2 mm/s crosshead speed) fitted on a TA-HDi Texture Analyzer (Stable Macro System, London, UK), following the procedure described by Sams et al. (1990). Reported WBSF data represent an average of the six measurements. Left breast samples and 30 right breasts (10 per treatment) were individually

vacuum-packed and frozen at –20 °C. Breast muscles were first thawed at 4 °C for 16 h, then left at rooom temperature until 4 °C was reached, and then dried and weighed. Thawing losses were calculated as percentage of the frozen weight. Then fillets were vacuum-packed again, cooked following the same above-mentioned procedure and weighed again to determine the cooking losses as percentage on the thawed weight. Mineral profile considered Calcium, Iron, Sodium, Potassium, and Phosphorus content. Inductively coupled plasma optical emission spectrometry (ICP-OES) was performed with Spectro Arcos (SPECTRO Analytical Instruments, GmbH, Kleve, Germany) after microwave digestion with Milestone rotor at 64-bar pressure, according to AOAC 2000 (Method no. 999.10). Data were expressed as mg/kg as is.

Table 4.1. Scheme used to evaluate *Pectoralis major* muscles.

Descriptors	**Wooden Breast**			
Consistency	Hard	Very hard		
Colour	Normal	Pale	Pink	Other
Bulge presence / diffusion	Not present	Cranial	Caudal	Longitudinally diffuse
Exudate presence / consistency	Not present	Fluid	Turbid	
Exudate colour	Clear	Gray	Yellow	
Haemorrhage presence / diffusion	Not present	Cranial	Caudal	Longitudinally diffuse
Haemorrhage width	Pinpoint	3 to 5 mm	≥ 5 mm	
White Striping presence / diffusion	Not present	Cranial	Caudal	Longitudinally diffuse
White Striping presence / width	Not present	≤ 1 mm	≥ 1 mm	

Sensory Analysis

After three months of frozen storage, the 30 right breast meat samples were subjected to a descriptive conventional profiling sensory analysis (as defined by ISO 13299, 2003), following the method suggested by Meilgaard et al. (1999) and O'Sullivan et al. (2003) on warm samples. The analysis was carried out in five consecutive days in order to avoid panel sensory fatigue, given the high number of samples to be tested. A panel of ten staff members of the MAPS Department was involved, after a 1.5-hour training session. During this session, several purchased and frozen breast samples were served to be evaluated as reference materials; all the descriptors to be used were developed, discussed, and selected, as intensity scores were assimilated (according to ISO 13299, 2003). Sensory analysis was carried out in a testing room with temperature set at 21 °C, neutral coloured wall and furniture, and standard lighting conditions. Permanent individual testing booths (76 cm wide × 51 cm depth) were arranged with two sets of six, facing each other across a central corridor and provided with a vertical sliding door to receive samples. Each day of analysis, six frozen half breasts randomly chosen between the three treatments were thawed (16 h at 4 °C), identified with a random three-digit

code,and cooked following the above-mentioned specification. Samples were served one by one whilst still warm and cut to have ten numbered and equally sized pieces, each corresponding to a specific panelist. First, breasts were cut in half in the thickness direction; then, a cranial, a medium, and a caudal portion were obtained from each half and the first two areas were further divided in two parts. The same ten people were employed in all five sessions; each person always tested the same breast portion throughout the single session and the whole test. Each assessor was equipped with plastic cutlery, plastic dish and glass, expectorant cup, water, unsalted crackers, and six paper ballots (one per sample), and had no knowledge about the samples history. Off-odours and off-flavours perceptions (overall intensity, rancid, fishy, wet cardboard), taste (sourness and bitterness), aroma intensity (as described by Rizzi et al., 2007), and texture (tenderness, juiciness, and fatness) were taken into account and ranked on a 150-mm unipolar continuous-line scale. Scores put nearby 0-mm indicated the lowest value for each considered attribute and scores put nearby 150-mm indicated the highest value (Table 4.2). Marks left on the line scales were measured with a ruler as distances from 0- mm anchors and the absolute values considered as scores.

Statistical Analysis

SAS (2004 version 9.3) statistical software package was used. Breast weight data were evaluated by using ANOVA and processed by choosing a general linear model that considered treatment as fixed effect (PROC GLM). Instrumental analyses (pH, colour traits, WBSF, thawing, and cooking losses) and mineral profile data were also analysed using the same model, with breast weight as covariate. A mixed model (PROC MIXED) was used to detect any myopathies influence on sensory analysis scores considering treatment and panelist as fixed and random effects, respectively. Single tasting was considered as experimental unit and sensory scores deviating more than ± 5 (mm or absolute value) were considered as outliers and removed from the dataset. Post hoc pairwise contrasts were evaluated by Bonferroni adjustments and three significance levels were assigned: $P < 0.05$; $P < 0.01$; and $P < 0.001$. In addition, Pearson correlation was performed considering $P < 0.05$ as significance level and principal component analysis (PCA) graphic art was obtained based on the treatment effect, after Varimax rotation.

Table 4.2. List of the sensory attributes and scales anchors.

	Scores	
Attributes	**0 mm anchor**	**150 mm anchor**
Off-odor		
Overall intensity	Extremely poor	Extremely strong
Rancid	Not rancid	Extremely rancid
Fishy	Not fishy	Extremely fishy
Wet cardboard	Not wet cardboard	Extremely wet cardboard
Other off-odours	Not perceived	Extremely strong
Off-flavor		
Overall intensity	Extremely poor	Extremely strong
Rancid	Not rancid	Extremely rancid
Fishy	Not fishy	Extremely fishy
Wet cardboard	Not wet cardboard	Extremely wet cardboard
Other off-flavors	Not perceived	Extremely strong
Taste		
Sourness	Not sour	Extremely sour
Bitterness	Not bitter	Extremely bitter
Aroma intensity	Extremely poor	Extremely strong
Texture		
Tenderness	Extremely tender	Extremely tough
Juiciness	Extremely dry	Extremely juicy
Fatness	Extremely poor	Extremely strong

3. Results and Discussion

As expected, breasts of the WSWB group were the heaviest ($P < 0.001$) (Figure 4.1) and exhibited the highest pH values ($P < 0.05$), which was strongly influenced by both treatment ($P < 0.05$) and breast weight ($P < 0.05$) (Table 4.3). In fact, previous results showed that WS, WB, and the simultaneous presence of the two degenerative conditions generally increase pH values (Petracci et al., 2013; Dalle Zotte et al., 2014, 2015b, Chapter 5; Mudalal et al., 2015). Le Bihan-Duval et al. (2008) obtained a high heritability for ultimate pH and glycogen storage (determined as glycolytic potential). A strong negative correlation between glycogen store and breast muscle weight was found; on the contrary, a strong positive correlation between breast weight and high ultimate pH of meat was noticed. It seems that the physiological functions in the lesion area are not normal. In addition, microscopic studies show that there are large areas of connective tissue in the muscle, meaning that the lesion itself causes swelling and therefore the increase in muscle volume. Fillets of the WSWB group also had the greatest a* and b* values ($P < 0.001$ and $P < 0.01$, respectively) whereas WS group did not differ from the N one; on the contrary, L∗ value did not differ among the three experimental groups. A work by Mutryn et al. (2015) found that fiber-type switching could occur in WB condition, thus

leading to a high expression of myoglobin genes and thus to redder muscles, which was detected in the present survey considering WSWB group and also by Dalle Zotte et al. (2014). Interestingly, Petracci et al. (2013) showed that WS condition was already sufficient to determine higher redness of meat compared to normal chicken breasts. Previous studies demonstrated that fillets affected by WS or WB are more yellow than non-affected ones, probably due to the strong fibrotic response (Petracci et al., 2013; Dalle Zotte et al., 2014). In our study, a different scenario was observed: WS alone did not affect the yellowness of the meat, whereas a probable additive effect of the simultaneous presence, WSWB, increased the b∗ value. As for L* value, other studies showed that WS, as well as WSWB conditions did not increase the paleness of the meat and this was in accordance with our study (Petracci et al., 2013; Mudalal et al., 2015). Cooked *Pectoralis major* muscle samples belonging to WSWB group were tougher ($P < 0.01$) than both N and WS ones (22.8 vs. 17.5 and 20.0 N, respectively). However, our findings were not confirmed by previous studies, as cooked WB (Dalle Zotte et al., 2014) and WSWB (Mudalal et al., 2015) meat samples exhibited the same toughness of the normal meat. However, meat samples analysed by Dalle Zotte et al. (2014) had been previously frozen for 3 months. In general, tenderness of meat is known to increase with freezing and thawing, as the formation of large extracellular ice crystals breaks down myofibrils, thus resulting in physical structure disruption (Leygonie et al., 2012). Differently, Mudalal et al. (2015) applied a different cooking procedure than that of the present study, thus possibly explaining different shear force results (Jeremiah and Gibson, 2003; Garcia-Segovia et al., 2007). WSWB breast meat samples displayed also the worst water cooking losses at 48 h *post-mortem* ($P < 0.01$) as well as after six months of frozen storage ($P < 0.05$), thus supporting previous results (Dalle Zotte et al., 2014; Mudalal et al., 2015; Petracci et al., 2015). Differently, thawing losses remained unaffected. In WS meat, the overall connective tissue increases to the detriment of the protein amount, which was found to be lower than normal meat (Petracci et al., 2014). Such structural change implies a marked reduction of actin and myosin contents, which are responsible of meat water holding capacity, in favour of collagen that does not bind water (Dalle Zotte et al., 2014; Petracci, et al., 2015). This replacement results in a significant impairment of protein functionality, which is not pH-dependent but linked to the muscle degeneration, since the greatest losses were seen in correspondence to the highest pH values (Petracci et al., 2013) (Table 4.3).

Figure 4.1. Differences in breast weights among NORMAL, WS and WSWB groups (P<0.0001)**.**

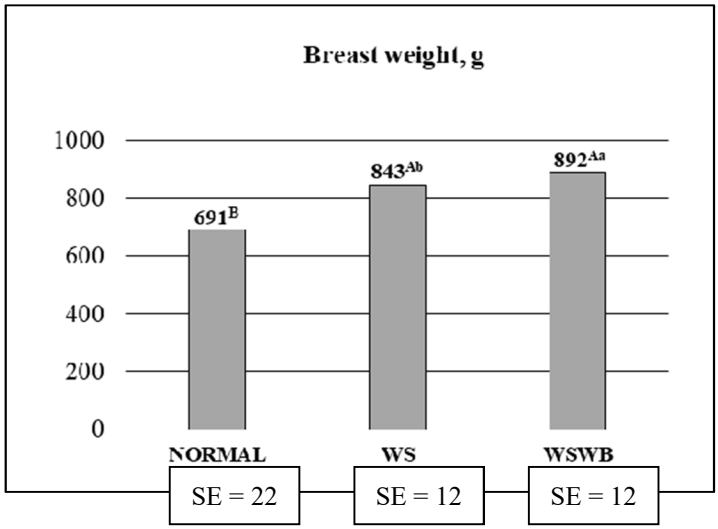


Table 4.3. Meat quality traits of *Pectoralis major* muscle.

Physical quality traits	Experimental groups			SE	*P*-value	COV Breast weight
	Normal	WS	WSWB			
pH [1]	5.92[b]	5.96[ab]	6.04[a]	0.03	0.01	0.01
L* [1]	56.0	55.8	55.9	0.51	0.97	0.57
a* [1]	-3.10[B]	-2.38[ABb]	-1.84[Aa]	0.18	0.0003	0.54
b* [1]	9.27[B]	10.3[ABb]	11.4[Aa]	0.36	0.002	0.05
WBSF (N)[1]	17.9[B]	20.7[B]	23.3[A]	0.82	0.0005	0.001
Cooking losses, 48 h *post mortem* (%) [1]	25.8[B]	27.7[B]	30.8[A]	0.89	0.001	0.38
Thawing losses (%) at 6 months [2]	5.43	6.02	6.44	0.49	0.47	0.47
Cooking losses (%) at 6 months [2]	22.9[b]	25.9[b]	29.1[a]	1.16	0.004	0.49

[a, b] : means within row with different superscripts differ at $P \leq 0.05$

[A, B]: means within row with different superscripts differ at $P \leq 0.01$ and $P \leq 0.001$

[1]: 108 right breast muscles (8 N, 50 WS, 50 WSWB)

[2]: 114 left breast muscles (10 N, 52 WS, 52 WSWB)

Table 4.4. Mineral profile of *Pectoralis major* muscle.

Minerals (mg/kg as is)	Experimental groups			SE	P-value	COV Breast weight
	N	WS	WSWB			
Ca	47.9	55.8	57.0	3.8	0.29	0.50
Fe	3.23[B]	4.45[Ab]	5.25[Aa]	0.18	<0.0001	0.39
Na	498[B]	566[AB]	631[A]	23	0.006	0.41
K	2,705[A]	2685[A]	2478[B]	42	0.002	0.12
P	2,033[A]	1984[A]	1822[B]	29	0.0003	0.70

[a, b]: means within row with different superscripts differ at $P \leq 0.05$

[A, B]: means within row with different superscripts differ at $P \leq 0.01$ and $P \leq 0.001$

[1]: 24 left breast muscles (8 N, 8 WS, 8 WSWB)

The WSWB fillets significantly differed from the N and WS groups also in terms of mineral profile: iron and sodium levels increased ($P < 0.001$ and $P < 0.01$, respectively), whereas potassium and phosphorus levels followed the opposite trend ($P < 0.01$ and $P < 0.001$, respectively) (Table 4.4). Recently Petracci et al. (2015) observed that WS condition reduced total ash percentage leading to a higher sodium concentration and lower potassium and phosphorus levels, however, this was not statistically revealed in our study. Even if no statistical difference was detected among groups, probably due to the small number of samples analysed, calcium content exhibited a growing trend going from N to WSWB meat samples (47.9, 55.8, and 57.0 mg/kg meat for N, WS, and WSWB, respectively). This partially supports the suggestion of Petracci et al. (2015), who hypothesized a connection between the two myopathies and an imbalance of calcium regulation. In fact, an uncontrolled and prolonged overload of intracellular calcium from extracellular sources or due to its mobilization from the sarcoplasmic reticulum can originate muscle tissue degeneration (Jones et al., 1984; Duncan and Jackson, 1987; Jackson, 1993; Sandercock and Mitchell, 2003, 2004). Muscle damage is triggered by the alteration of the cell membrane integrity (Mitchell, 1999) mediated by Ca2+- activated proteases and lipases, such as phospholipase A2 (Jackson et al., 1984; Jackson, 1993; Sandercock and Mitchell, 2003). Sandercock and Mitchell (2004) hypothesized that calcium uptake into muscle cells might be mediated by an increased muscle sodium level, which was observed also by Sandercock et al. (2009) in chicken selected for broiler traits. Therefore, sodium homeostasis disturbances may have a significant role in the development of cell injury, through the involvement of the Na+/Ca2+ exchanger (Sandercock and Mitchell, 2004). In dogs, skeletal muscle cell damage is also associated to moderate phosphorus depletion, which results in abnormally increased intracellular sodium and chloride ions levels, along with the decline of normal potassium concentration and transmembrane potential. The triggering factor seems to be a lowered synthesis of adenosine triphosphate (ATP); in effect, the lack of energy may interfere in the activity of the Na+/K+ - ATPase in maintaining a high concentration of potassium ions within the cell. Phosphorus decrease may also result in greater myofiber permeability to sodium and compromised sarcolemma integrity, as consequences of lowered ATP availability for sarcolemma metabolic processes as well as the depletion or alteration of certain lipid compounds (Fuller et al., 1976). Chronic WB may be linked to localized hypoxia, oxidative stress, as well as the possible presence of muscle fiber-type switching, as demonstrated by the work of Mutryn et al. (2015) on RNA-sequence analysis. The last phenomenon consists in the change from fast to slow twitch fibers in response to muscle degeneration, with the consequent unexpected high expression of myoglobin (Mutryn et al., 2015), which could explain the greatest iron content and a* color value in our WSWB breasts.

Table 4.5. Sensory analysis scores of *Pectoralis major* muscle.

Attributes [1]	Experimental groups			SE	*P*-value
	Normal	WS	WSWB		
Off-odours					
Overall intensity	1.86^{b}	2.70^{a}	2.20^{ab}	0.31	0.02
Rancid	0.84	0.91	0.98	0.23	0.69
Fishy	0.36	0.29	0.42	0.14	0.55
Wet cardbox	0.74	0.84	0.76	0.17	0.83
Other off-odours	0.00	0.00	0.00	-	-
Off-flavours					
Overall intensity	1.57	1.48	1.76	0.28	0.59
Rancid	0.79	0.80	1.03	0.25	0.30
Fishy	0.33	0.24	0.38	0.16	0.57
Wet cardbox	0.67	0.80	0.68	0.21	0.73
Other off-flavours	0.64	0.45	0.62	0.23	0.53
Taste					
Sourness	2.06	1.74	1.71	0.33	0.43
Bitterness	1.56	1.98	1.82	0.37	0.35
Aroma intensity	4.76	4.45	4.14	0.57	0.11
Texture					
Toughness	4.70^{B}	5.56^{AB}	6.22^{A}	0.40	0.004
Juiciness	5.14	4.98	4.58	0.44	0.33
Fatness	1.26	1.32	1.25	0.39	0.87

$^{a, b}$: means within row with different superscripts differ at $P \leq 0.05$

$^{A, B}$: means within row with different superscripts differ at $P \leq 0.01$and $P \leq 0.001$

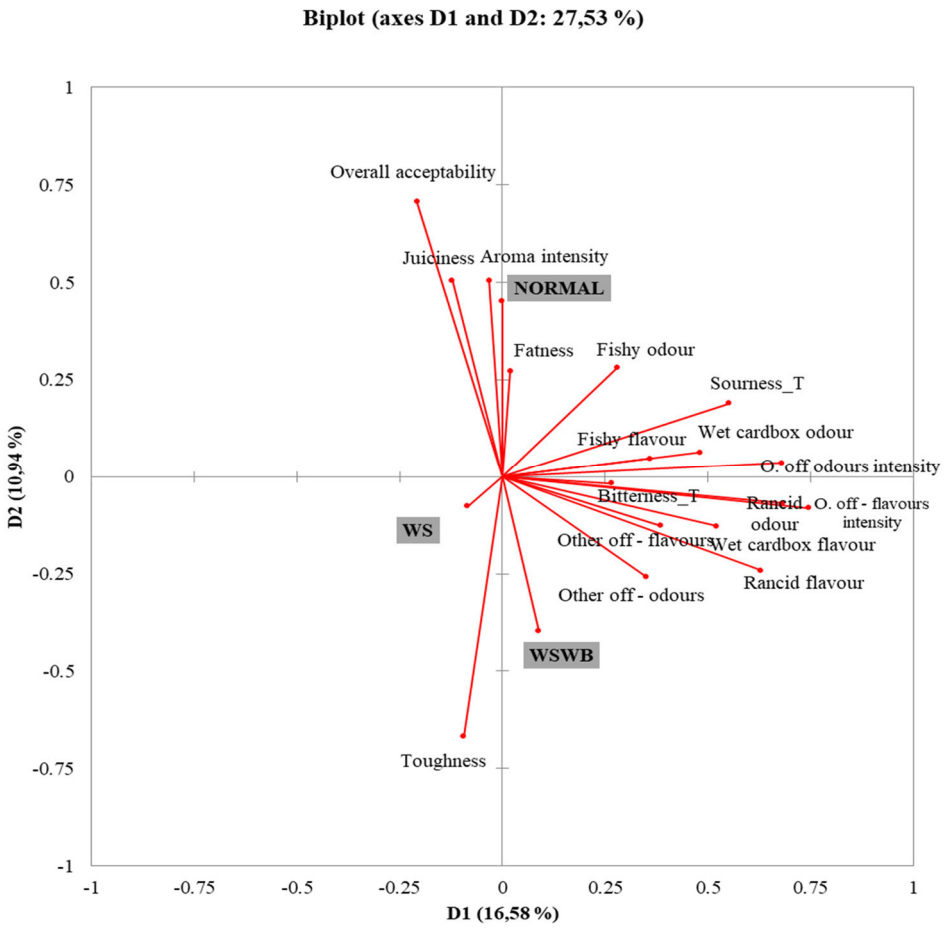
[1]: 30 right breast muscle (10 N, 10 WS, 10 WSWB)

The sensory profile analysis revealed that off-odours were more intense in fillets of the WS group compared to the N ones, whereas WSWB fillets reached inter-mediate scores ($P < 0.05$). Nevertheless, panelists did not find any difference concerning specific off-odour perception among groups (Table 4.5). Even if no statistical difference was observed, panelists seemed to perceive a certain decreasing trend going from N to WSWB meat samples for sourness, aroma intensity, and juiciness. Such tendencies were opposite to that of pH value (Table 4.3), probably because pH is a key factor in sourness perception. Therefore, it is involved in aroma perception (Madruga and Mottram, 1995; Meynier and Mottram, 1995) and the experience of juiciness (Hoffman et al., 2007). Regarding overall and specific off-flavour intensity, bitterness, juiciness, and fatness, neither differences nor trends were detected among the three groups. WSWB group received the highest toughness scores ($P < 0.01$), thus supporting the greatest instrumental shear force values previously discussed. PCA was performed to evaluate the relationships among sensory variables. The first two principal components accounted for 27.5% of the total variance (Figure 4.2). The first PC (16.6% of the total var-

iance) was positively loaded by overall off-odour intensity (0.68), rancid odour (0.68), overall off-flavour intensity (0.75), and rancid flavour perception (0.63). The second PC (10.9% of the total variance) was positively loaded by juiciness (0.51), but negatively loaded by toughness (-0.67). The PCA plot shows that the WSWB condition is the most positively linked to toughness (0.16). It is worthy to be noticed that the fatness attribute is located in the opposite position from WS and WSWB but near the N group. This could be explained by considering that the slight fat perception is related to juiciness (0.31). Given that the visual aspect of WS affected meat led to product rejection at purchase (Kuttappan et al., 2012b), the present work tried to describe a sensory pro-file of the cooked meat, avoiding the negative influence of raw meat appearance prior to tasting. Macroscopic lesions characterizing WS and WB were not detected after cooking; despite this, the hedonic quality of WSWB meat was lower than that of normal samples due to a harder texture feeling in the mouth.

In our material, it was impossible to find breast muscles affected only by WB myopathy, contrary to previous findings of Mudalal et al. (2015). In addition, WS prevalence appears to have increased dramatically in a few years when comparing to the study of Petracci et al. (2013) and that of Lorenzi et al. (2014), Dalle Zotte et al. (2015b) (Chapter 5) and Russo et al. (2015). In conclusion, this investigation demonstrated that the simultaneous presence of WS and WB exerted an additional detrimental effect on technological traits, nutritional quality, and toughness perception respect to the single lesions individually considered. A strict connection and common causative basis may be hypothesized between the two conditions, even if they still need to be elucidated. Thus, further studies should elucidate the link existing between the two myopathies and their development involving biochemical and genic analysis, to better understand the pathological pathways.

Figure 4.2. Plot of the sensory attributes based on the two variables that mainly explain the PCA model variability: overall off-odours intensity (D1) and rancid odour (D2).



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CHAPTER 5

Impact of coccidiosis control program and feeding plan on white striping prevalence and severity degree on broiler breast fillets evaluated at three growing ages

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ABSTRACT

This study investigated the impact of 2 coccidiosis control systems (vaccine vs anticoccidial) and 2 feeding plans (standard energy vs low energy content, the latter supplemented with threonine and enzymes in the second half of the production cycle) on white striping (WS) prevalence and severity in chicken broiler breasts at commercial slaughter age (51 d). The age of lesion onset was also investigated with the sacrifice of 80 chicks at 12, and 80 chicks at 25 d of age. Seven hundred and twenty ROSS 708 strain male chicks were divided into 4 groups: a non-vaccinated group fed with standard diet (CONTROL); two groups vaccinated against coccidiosis but fed either a standard diet (VACC) or a low-energy diet supplemented with threonine and enzymes (VACC–LE plus); and a fourth group fed a standard diet containing anticoccidial additive except during the finishing period (COX). After live performance, yields, and fillet pH were measured, the breasts were weighed and scored as level 0 (no WS), level 1 (moderate WS), and level 2 (severe WS) at each of the 3 ages; data were covariate for slaughter weight. The results suggest an ameliorative effect of coccidiosis control systems when compared to the control group in terms of live weight, breast yield, and whole breast weight, with heavier fillets characterized by higher pH values. WS appeared at 25 d of age with an average prevalence of 11.5% and with lesions of moderate severity. There were no statistically significant differences due to the experimental treatment at this age. At commercial slaughter age, total average prevalence was 96%, with COX birds showing higher level 2 prevalence (77.6%). This could be related to the higher slaughter weight reached by the COX group ($P < 0.001$) and the treatment effect ($P < 0.01$) that probably adds to the effect of live weight. Diet had no effect on overall live performances of VACC-LE plus chickens, which were similar to those of the VACC group.

Key words: chicken, white striping, coccidiosis control, feeding plan.

1. Introduction

In recent decades, increased consumer demand has required producers to intensify production output, mainly through high-energy diets (Kuttappan et al., 2012a) and an intensive selection of strains exhibiting faster growth with higher breast yields; an increase in the incidence of *Pectoralis major* myopathies has however been observed over this same period (Petracci et al., 2013a). White Striping (WS), an emerging meat quality issue, is a muscle defect described as the appearance of macroscopic white striations running parallel to the direction of muscle fibers in broiler breast fillets first pointed out by Bauermeister et al. (2009) and Kuttappan et al. (2009). More recently, Kuttappan et al. (2013a) noticed that these lesions could exhibit a variable degree of severity and suggested a score based on the size and distribution of white striations on the breast surface. Fillets with apparently no lesions were classified as normal; those with easily observed striations generally having a thickness less than 1 mm were scored as moderate; and breasts with white striations thicker than 1 mm covering a greater surface area were scored as severe. Macroscopic WS prevalence ranges from 12 to over 50% (Kuttappan et al., 2009, 2012a; Petracci et al., 2013b). Poultry processors are obliged to downgrade WS breasts and process them further instead of being able to offer them for fresh retailing, at great economic loss (Petracci et al., 2013b). Indeed, visual appearance is the most important attribute available for consumers to assess the quality of a meat product at purchase, and WS may decrease breast fillet acceptability (Kuttappan et al., 2012b). Histologically, a widespread degenerative myopathy associated with chronic lesions characterizes severe degrees of WS. Hypertrophy, cell changes (appearance of giant fibers, small size, rounded fibers, internalization of nuclei, loss of cross striations), and tissue damage with degeneration and necrosis of fibers (appearance of inflammatory cell infiltration, fibrosis, lipidosis) have all been observed (Kuttappan et al., 2013a; Petracci et al., 2013a). Several authors associate WS occurrence with higher live weight at older ages (Bauermeister et al., 2009) or the higher growth rate of birds with heavier breasts (Kuttappan et al., 2009, 2013a). Genotype seems to play a key role, given that high breast yield strains generated thicker fillets with higher prevalence of both moderate and severe WS than standard breast strains (Kuttappan et al., 2012a). The implementation of diets with higher energy levels might also play a role, since growth rate and breast meat yield are both higher in birds fed this type of diet than those fed reduced energy diets (Kuttappan et al., 2012a). Breast muscle portions are not affected with the same intensity. The severity of WS is greater toward the cranial end of the ventral surface, where the fillet exhibits maximum thickness, than the caudal portion (Kuttappan et al., 2013a), probably because convexity or stretching is greater in this region. Moreover, Brewer et al. (2012) found a high correlation ($r = 0.81$) between fillet weight and cranial thickness. This finding suggests greater WS occurs in con-

junction with heavier or thicker fillets (Kuttappan et al., 2013c). White and red muscles are notsubject to the same WS occurrence; Kuttappan et al. (2013c) found that *Pectoralis major* and *Iliotibialis* presented a higher incidence than *Gastrocnemius*, suggesting the greater susceptibility of white glycolytic fibers. Hoving-Bolink et al. (2000) suggested that genetic selection for chickens with higher breast yield might have adversely affected blood supply; indeed, the lower capillary density might have resulted in a reduced supply of nutrients, oxygen, and the removal of catabolites, leading to tissue damage. Moreover, Kuttappan et al. (2013a) suggested that severe degrees of WS (associated with faster bird growth rate) make muscles prone to developing overstretching, or ischemia, resulting in tissue damage and attempted reparative response. Not all potential causes of WS lesion have been fully investigated. To date, no studies have explored the link between anticoccidials and WS, even though the toxicity of coccidiostats in skeletal muscle, visible as focal degeneration and necrosis, has already been reported in broilers (Sandercock and Mitchell, 2003; Chapman et al., 2010), turkeys (Markiewicz et al., 2014), and other species (Novilla, 1992). Our study was conducted to investigate certain aspects not yet considered, such as the impact of 2 coccidiosis control programs (vaccine vs anticoccidial additive) and feeding plan on WS occurrence. This also implied considering the relationship between anticoccidial drug administration and anticoccidial vaccination of chickens fed diets with different energy density and bioavailability of nutrients and WS prevalence. Moreover, the observation of the breasts of chickens at different ages (12, 25 and 51 d) was intended to identify the age of onset of WS. This study will hopefully make a significant contribution to the identification or exclusion of factors leading to WS.

2. Materials and Method

Experimental Design and Management

The experiment was performed in a broiler unit consisting of a central corridor and 12 pens on each side. In this study, 720 male 1-day-old chicks of ROSS 708 hybrid were individually tagged at wing level, randomly divided into 4 experimental groups (CONTROL, VACC, VACC-LE plus, COX) of 180 birds each, and housed in floor pens until 51 d of age. They were vaccinated for Newcastle disease, infectious bronchitis, and Marek's disease at the hatchery. On the fourth day of life, the vaccine against coccidiosis Paracox-5 (MSD Animal Health S.r.l., Segrate, Milano, Italy) was administered in drinking water to chicks in groups VACC and VACC-LE plus, following the manufacturer's instructions. The rearing period was divided into 4 feeding phases: first period (0 to 12 d of age), second period (12 to 25 d of age), third period (25 to 42 d of age), and fourth period (42 to 51 d of age). Animals received different feeding plans: the non–vaccinated groups (CONTROL and COX)

and one vaccinated group (VACC) were fed standard diets through-out the entire rearing period, whereas group VACC– LE plus was fed low energy diets (C3 and C4) supplemented with threonine (0.6 and 0.4 g/kg, respectively), xylanase (2 g/kg) and QuantumTM phytase 2500 D (6- phytase) (EC 3.1.3.26) (0.3 g/kg) enzymes in the last 2 periods. The COX diet contained anticoccidial additive except during the finishing period. Feed and water were provided for ad libitum consumption and feed was provided daily in the feeders. During the first and the second periods, birds were reared in 3 m^2 pens at a stocking density of 20 birds/m^2 (60 birds/pen) and divided into 12 pens (3 replicates/treatment). At the first and the second feeding changes (12 and 25 d of age), 2 intermediate slaughterings were performed with the sacrifice of 80 animals (20 per treatment) each (160 slaughtered overall) by cervical vertebrae dislocation as specified by Reg. (CE) No. 1099/2009. At 26 d of age, the remaining chickens in each pen were equally allotted into two 3 m^2 pens, in this way doubling the number of pens per treatment (6 replicates/treatment) with a stocking density at 51 d of kg/m^2 (an average number of 22 birds/pen, 7.3 birds/m^2). All pen floors were covered with beech shavings and equipped with a 120 cm circumference feeder (to ensure 4.4 cm of front space) and a 40 cm diameter automatic bell drinker. Photoperiod length was set to Council Directive 2007/43/EC recommendations. Environmental conditions were recorded daily by measuring the minimum and maximum temperatures inside pens at ground level and in the poultry house central hallway, where relative humidity was also measured. Light intensity was monitored at bird head height using a digital illuminometer (4 in 1 Multi-Function Environment Meter Lafayette; Product code: DT-8820). Animals were monitored twice daily throughout the study to assess availability of feed and water, mortality, and any potential conditions of morbidity. At every feeding change (12, 25, and 42 d of age) and at the end of the rearing period, all birds were individually weighed. Feed intake (FI) was recorded by weighing the feed provided and subtracting the residuals. Average daily gain (ADG), FI, feed conversion ratio (FCR = kg of feed consumed/kg of weight gain), and mortality were determined for each feeding phase for the entire rearing period with the pooling of the first 2 (0 to 25 d) and the last 2 (25 to 51 d) periods.

The study was approved by the Ethical Committee for Animal Experimentation of the University of Padova, Italy (Project number 17/2014 approved in May 2014, Prot. No. 71360). Birds were handled according to the principles stated in EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

Feeding Plan

Nutega (Nuevas Tecnologias de Gestion Alimentaria S.L., Madrid, Spain) formulated the diets according to Ross 708 nutrient specifications. During the first 2 feeding phases (0 to 12 and 12 to 25 d of age), birds in CONTROL, VACC and VACC-LE plus groups received the same standard diet indicated as B1 (Starter) and B2 (Grower), respectively. Birds from COX group received A1 (Starter) and A2 (Grower) diets with the same chemical composition as the B1 and B2 diets containing the anticoccidial Maxiban G 160 premix in a 0.5 kg/ton dose (Elanco Animal Health division of Eli Lilly & Co., Indianapolis, IN). During the third feeding phase (25 to 42 d of age), CONTROL and VACC groups received diet B3, whereas COX group received diet A3 enriched with anticoccidial Elancoban 200 premix in a 0.5 kg/ton dose (Elanco Animal Health division of Eli Lilly & Co., Indianapolis, IN). VACC-LE plus group received a finishing diet C3 characterized by lower energy content (4,560 kcal gross energy (GE)/kg) than the A3 and B3 diets (4,638 and 4,659 kcal GE/kg, respectively). During the finishing period (42 to 51 d of age), CONTROL, COX, and VACC groups received a standard Finisher diet (4,639 kcal GE/kg), whereas VACC-LE plus birds continued receiving a low-energy diet (C4; 4,551 kcal GE/kg) (Tables 5.1 and 5.2). As above stated, in the periods 3 and 4 the diets C3 and C4 were also supplemented with threonine, xylanase, and phytase, with the purpose of maximizing nutrient bioavailability to birds, and to verify if the combination with a low energy diet could be beneficial for WS prevalence reduction.

Table 5.1 Feeding plan.

	Treatments (T)			
	CONTROL	COX	VACC	VACC-LE plus
Period 1 (0 to 12 d)	B1	A1	B1	B1
Period 2 (12 to 25 d)	B2	A2	B2	B2
Period 3 (25 to 42 d)	B3	A3	B3	C3
Period 4 (42 to 51 d)	Finisher	Finisher	Finisher	C4

Chemical Analysis of Feed Samples

Experimental diets were sampled, finely ground, and analyzed using AOAC (2002) methods to determine the concentrations of dry matter (DM; Method no. 934.01), ash (Method no. 967.05), crude protein (CP; Method no. 2001.11), ether extract (extraction with diethyl ether reagent, Method no. 920.39), crude fiber (CF; Method no. 978.10), and starch (amyloglucosidase-alpha-amylase, Method no. 996.11). Neutral detergent fiber (NDF) content was analyzed according to Mertens (2002), and

minerals (Ca, P, K, and Na) were quantified by ICP-OES (Spectro Ciros Vision EOP) after microwave digestion (AOAC 2000, Method no. 999.10). Data were reported as g/kg as fed. GE and Metabolizable energy (ME) values were calculated on the basis of equations in the European Tables (Janssen, 1989).

Slaughter and Processing of Birds

In order to detect the onset of WS in the breast muscle (*Pectoralis major*) and to study its evolution, randomly selected chickens were weighed and sacrificed as described above at 12 and 25 d of age, and their whole breasts (*Pectoralis major* and *Pectoralis minor*) were dissected and weighed. *Pectoralis major* muscle was macroscopically observed to score as level 0 (no WS), level 1 (moderate WS), and level 2 (severe WS) according to Kuttappan et al. (2013a), whose ranking system is based on the size and distribution of white stripes on the fillet surface: level 0 when white stripes were visually absent; level 1 when white stripes were ≤1 mm thick, and level 2 when white stripes were ≥1 mm thick and covered a large surface of the fillet. Ultimate pH (pHu) was determined in the cranial and caudal portions of the fillet using a FG2-FiveGo portable pH-meter (Mettler Toledo, Greifensee, Switzerland). At 51 d of age, the remaining birds were individually weighed, subjected to a 12 h total feed withdrawal, and processed in an authorized commercial slaughterhouse using electrical stunning. Carcasses (obtained by removing head, neck, shanks, and abdominal fat from bled, plucked, and eviscerated birds) were chilled, stored at 4 °C, and transported to a commercial processing plant where the chilled carcasses and whole breasts were weighed to compute carcass and breast yields, pHu was measured on the cranial and caudal portions of the fillets, and fillets were subjected to visual WS scoring as above.

Statistical Analysis

A SAS (2004-version 9.3) statistical software package was used. Individual live weight (LW) and ADG data were evaluated by using ANOVA and processed by choosing a mixed model that considered the pen as a random effect and treatment as a fixed effect (PROC MIXED). Slaughter yields, breast yields, and pHu were also analyzed using the same mixed model, with slaughter weight as covariate (COV). FI and FCR data, calculated at pen level, were processed through a one-way ANOVA with treatment as a fixed effect (PROC GLM). WS prevalence percentages were processed through ANCOVA analysis to detect possible treatment and live weight effects. WS scores were analysed through the non-parametric Kruskal-Wallis test (PROC NPAR1WAY). Post hoc pairwise contrasts were evaluated by Bonferroni adjustments and three significance levels were assigned: ∗: $P < 0.05$; ∗∗: $P < 0.01$; and ∗∗∗: $P < 0.001$. The minimum number of birds required for this study was

defined as 136 animals per group, using the formula:

$$(1.962(P_{att}\ (1 - P_{att})))/D^2$$

(where P_{att} is the expected prevalence and D is the absolute precision) and assuming an estimated prevalence of about 80%, an absolute precision of 7%, and a confidence level of 95%. Considering an average mortality of around 5%, the decision to rear 180 chicks per group (a total number of720) was made. The purpose of the first 2 slaughters (at 12 and 25 d of age) was to detect WS onset. For this reason, the necessary sample size was assessed as 18 animals per group when assuming a prevalence ≥ 15% and a confidence level of 95% (Cannon and Roe, 1982).

Table 5.2 Ingredients and nutrient composition of experimental diets.

	Starter	Grower			Finisher	
Ingredients (g/kg as fed)	A1-B1	A2-B2	A3-B3	C3	C4	Finisher
Corn 7.7%	536	627	653			680
Corn 7.9%				682	705	
Soybean meal solv extr 47.5%	361	303	270			244
Soybean meal solv extr 47%				246	226	
Soybean/Sunflower oil	30.0	35.8	47.4			47.4
Soybean oil				36.0	35.0	
Gluten 56	30.0					
Calcium carbonate	14.3	11.2	10.4	10.1	10.2	9.9
Monocalcium phosphate 22.5/16	13.6	9.50	8.30	10.3	8.90	8.00
Sodium bicarbonate	1.10	1.40	0.70	0.70	0.70	0.80
Salt	2.10	1.90	2.40	2.40	2.40	2.30
DL-Methionine 99%	3.50	3.20	2.20	2.10	1.90	2.00
L-Lysine HCL 98%	2.80	2.00	0.70	2.10	1.80	0.90
L-Threonine 98%	0.60	0.50		0.60	0.40	
Vitamin[1] and mineral[2] premix	5.00	5.00	5.00	5.00	5.00	5.00
Xylanase				2.00	2.00	
Phytase-2500				0.30	0.30	
Monteban 100 premix[3] (A1 and A2 only)	0.0005	0.0005				
Elancoban 200 premix[4] (A3 only)			0.0005			
Analysed nutrient composition (g/kg)						
Dry matter (DM)	897	897	898	898	896	912
Ash	52.5	43.5	41.5	43.0	39.0	40.0
Organic matter[5] (OM)	844	854	856	855	857	872
Crude protein (N x 6.25) (CP)	240	197	179	172	164	168
Ether extract (EE)	48.5	54.0	69.0	58.0	56.0	70.0
Crude fibre (CF)	20.0	21.0	21.5	16.0	14.0	22.0
NDF	108	107	112	108	126	110
Starch	349	403	424	425	446	436
Non-nitrogenous extracts[6]	536	582	587	608	624	613
NNCC[7]	496	551	566	575	568	594
Ca	10.8	8.55	7.40	7.80	7.90	7.30
P	6.95	5.85	5.05	5.60	5.50	4.70
Ca/P	1.55	1.46	1.47	1.40	1.43	1.55
K	9.55	8.90	7.85	7.80	7.60	7.30
Na	1.40	1.30	1.35	1.40	1.30	1.40
GE (kcal/kg) [8]	4,581	4,582	4,649	4,560	4,551	4,639
ME (kcal/kg) [8]	3,055	3,156	3,258	3,130	3,148	3,283

[1] Provided the following per kilogram of diet: E672 vitamin A, 12,500 I.U.; E671 vitamin D3, 5,000 I.U.; 3a700 vitamin E, 30 mg (alfa
– tocopheryl acetate); vitamin K, 5 mg; vitamin B1, 3 mg; vitamin B2, 6 mg; vitamin B6, 3 mg; vitamin B12, 0.03 mg; vitamin PP, 40 mg; pantothenic acid/calcium D - pantothenate, 20 mg; biotin, 0.2 mg; folic acid, 0.75 mg

[2] Provided the following per kilogram of diet: Fe, 78 mg; I, 0.925 mg; Cu, 20 mg; Mn, 71.15 mg; Zn, 75 mg; Se, 0.27 mg (as E1 ferrous sulphate monohydrate, E2 potassium iodide, E4 cupric sulphate pentahydrate, E5 manganese oxide, E6 zinc sulphate monohydrate, E8 sodium selenite)

[3] Anticoccidial additive ingredients (%): Narasin sodium, 10; diluent (may include rice hulls, corn grits, or similar), 85 – 90; anti- dusting oil, 0-5. Elanco Animal Health division of Eli Lilly & Co., Indianapolis, IN

[4] Anticoccidial additive ingredients (g/kg of additive): Monensin granulated, 930 (equiv. Monensin sodium: 200); antidusting oil, 20; rice hulls or limestone granular, 50. Elanco Animal Health division of Eli Lilly & Co., Indianapolis, IN

[5] Organic matter content = DM – Ash

[6] Non-nitrogenous extracts content = 100 – (Ash + CP + EE + CF)

[7] NNCC: non-nitrogenous cellular content = OM – (CP + NDF)

[8] Estimated according to Janssen (1989)

3. Results and Discussion

Diet Formulation

Dietary ingredients and formulation are reported in Table 5.2. Diets administered to the 4 experimental groups during period 1 and period 2 (A1 and B1; A2 and B2, respectively) were characterized by a similar formulation. The formulation of diets A3, B3 (of period 3), and Finisher (of period 4) differed from that of diets C3 and C4 (of period 3 and 4, respectively), which were low-energy diets. Diets with different GE levels differed in corn and soybean with different protein content, oil source, and oil content. Diet nutrient content is presented in Table 5.2. The differences in the ingredients of diets fed at period 3 and period 4 regarded nutrient content in terms of CP, EE, CF, and ME. As intended, the GE of diets A3 and B3 was higher than that of C3, whereas ME estimation provided a similar difference (3,258 vs 3,130 kcal/kg, respectively). Finisher diet differed from C4 in DM, organic matter, CP, EE, and CF, and therefore led to higher GE and estimated ME (3,283 vs 3,148 kcal/kg, respectively). Consequently, the differing ME content between standard and low-energy diets was expected to produce differences in bird growth rates.

Live Performances

At 12 d of age, CONTROL group broiler live weight was lower than that of animals in the other experimental groups ($P < 0.001$), whereas at 25 d of age, CONTROL and COX group birds were heavier than the vaccinated groups ($P < 0.05$; Table 5.3). At 42 d of age, CONTROL group chickens again had the lowest live weight, whereas COX and VACC-LE plus groups had the highest ($P < 0.001$). At final slaughter (51 d of age), COX and VACC-LE plus birds were still heavier (3,667 and 3,672 g, respectively) than CONTROL group birds (3,489 g; $P < 0.01$). In period 1 (0 to 12 d of age), CONTROL group birds exhibited an unfavorable FCR (1.21) compared with those in the other experimental treatments (1.11, 1.13, 1.10 for COX, VACC, and VACC-LE plus, respectively; $P < 0.01$) due to their significantly ($P < 0.001$) lower ADG. During period 2 (12 to 25 d of age), both CONTROL and COX groups showed higher ADG than VACC and VACC-LE plus groups ($P < 0.001$), together with a favorable FCR ($P < 0.001$). In period 3 (25 to 42 d of age), although no difference in terms of FI emerged among groups, COX and VACC-LE plus birds had higher ($P < 0.05$) ADG (102 and 105 g/d, respectively) than those of CONTROL and VACC groups (80.3 and 95.2 g/d, respectively). During the finishing period (42 to 51 d of age), CONTROL group chickens provided the best live performance, partially recovering the worst performance recorded in previous periods. In this fourth period, vaccinated groups had improved live performance and ranked in between the other 2 groups, whereas the ADG of the COX birds declined dramatically. Overall, during

the first 2 feeding periods, non-vaccinated groups showed higher ADG (49.4 and 50.7 g/d for CONTROL and COX, respectively) and favorable FCR (1.42 for both treatments) than VACC and VACC-LE plus groups (46.4 and 47.2 g/d; 1.47 and 1.48, respectively; $P < 0.001$). This evidence supports the depressive effect of anticoccidial vaccine on both FI and live performance in the early growing phase. Only VACC birds experienced FI depression however, whereas VACC-LE plus birds appeared to have been less affected (64.4 vs 65.6 g/d; $P < 0.05$). During the last 2 feeding periods (25 to 51 d of age), coccidiosis control systems proved capable of improving live per-formance, with the highest ADG and the most favorable FCR exhibited by VACC-LE plus birds. Considering the entire rearing period (0 to 51 days), there were no differences for FI and FCR among groups, but higher ADG was observed in COX and VACC-LE plus groups (71.1 and 71.2 g/d, respectively) than in the CONTROL group (67.6 g/d; $P < 0.01$). Treatments did not affect animal health, and the average mortality recorded during the trial was 5% (Table 5.3). Under comprehensive analysis, the data obtained suggest an overall ameliorative effect of coccidiosis control systems on live performance. The favorable effect the carboxylic ionophores (Narasin and Monensin) have on growth was confirmed. Indeed, during their period of use as additives, these polyether antibiotics ensured an intestinal integrity that contributed to improved ADG and FCR. During period 4, COX group underwent anticoccidial withdrawal and, as expected, exhibited lower ADG and FCR. Despite the depressive effect on feed consumption observed in VACC and VACC–LE plus birds during period 2 which also negatively affected ADG and FCR, the 2 coccidiosis control pro grams (VACC and COX) exhibited similar live performance when the rearing period was considered in its entirety. These results support previous studies (Govoni et al., 1987; Shirley and Long, 1990 and Shirley, 1993; Williams and Gobbi, 2002) in which no statistically significant differences in terms of commercially important performance criteria were found between vaccinated birds and those given anticoccidial treatment. Chickens fed low energy diets (VACC-LE plus) during periods 3 and 4 did not fill the energy gap by higher FI. Nevertheless, birds in this experimental group expressed live weight, ADG, and FCR similar to those fed higher energy diets (COX and VACC). VACC-LE plus birds might have benefited from the dietary inclusion of enzymes (phytase and xylanase), more abundant lysine supplementation, and the inclusion of threonine in the last 2 rearing periods, thereby increasing the digestive utilization of nutrients and promoting protein synthesis. It is therefore likely that the ME of C3 and C4 was effectively higher than estimated.

Table 5.3. Live performances.

Periods	Treatments (T)				SE	P
	CONTROL	COX	VACC	VACC – LE plus		
Live weight, LW (g)[1]						
12 d	370^{B}	409^{A}	402^{A}	417^{A}	5	***
25 d	1277^{ABa}	1308^{A}	1199^{C}	1222^{BCb}	11	***
42 d	2635^{C}	3033^{A}	2826^{B}	2992^{A}	26	***
51 d	3489^{b}	3667^{a}	3593^{ab}	3672^{a}	36	**
Average daily gain, ADG (g/d)[1]						
0 - 12 d	27.4^{B}	30.6^{A}	30.0^{A}	31.2^{A}	0.4	***
12 - 25 d	69.5^{A}	69.2^{A}	61.4^{B}	62.0^{B}	0.7	***
25 - 42 d	80.3^{C}	102^{ABa}	95.2^{Bb}	105^{A}	1.3	***
42 - 51 d	94.9^{Aa}	66.7^{C}	85.4^{ABb}	75.1^{BCc}	2.1	***
0 - 25 d	49.4^{A}	50.7^{A}	46.4^{B}	47.2^{B}	0.4	***
25 -51 d	85.1^{Bb}	90.2^{ABa}	91.5^{A}	94.7^{A}	1.2	***
0-51 d	67.6^{b}	71.1^{a}	69.6^{ab}	71.2^{a}	0.7	**
Feed intake, FI (g/d)						
0 - 12 d [2]	32.8	33.7	34.2	34.5	0.4	NS
12 - 25 d [2]	100^{AB}	103^{Aa}	96.7^{B}	98.5^{Bb}	0.8	**
25 - 42 d [3]	204	212	207	203	4.8	NS
42 - 51 d [3]	196^{a}	168^{b}	181^{ab}	178^{ab}	6.0	*
0 - 25 d [2]	65.5^{a}	67.2^{a}	64.4^{b}	65.6^{a}	0.5	*
25 - 51 d [3]	202	200	199	195	4.1	NS
0 - 51 d [3]	127	126	126	124	2.0	NS
Feed conversion ratio, FCR						
0 - 12 d [2]	1.21^{Aa}	1.11^{B}	1.13^{Bb}	1.10^{B}	0.01	**
12 - 25 d [2]	1.44^{B}	1.48^{B}	1.55^{A}	1.58^{A}	0.01	***
25 - 42 d [3]	2.51^{A}	2.08^{B}	2.14^{B}	1.92^{B}	0.07	***
42 - 51 d [3]	1.97^{Bc}	2.52^{Aa}	2.12^{ABbc}	2.39^{ABab}	0.09	**
0 - 25 d [2]	1.42^{B}	1.42^{B}	1.47^{A}	1.48^{A}	0.01	***
25 - 51 d [3]	2.37^{A}	2.20^{AB}	2.17^{AB}	2.06^{B}	0.05	***
0 - 51 d [3]	1.97	1.92	1.90	1.84	0.04	NS
Mortality (%)[4]						
0 - 12 d[5]	1.09	1.11	0.0	0.56	1.19	NS
12 - 25 d[5]	0.64	0.60	1.26	0.62	1.62	NS
25 - 42 d[6]	1.49	2.17	4.35	2.08	4.94	NS
42 - 51 d[6]	2.11	4.49	0.69	1.48	3.11	NS
0 - 25 d[5]	1.65	1.65	1.11	1.11	1.76	NS
25 - 51 d[6]	3.60	6.52	5.04	3.57	5.78	NS
0 - 51 d[6]	4.39	6.65	5.08	3.89	4.91	NS

[a, b] Means within the same row followed by different lowercase superscript letters differ $P \leq 0.05$ (*)
[A, B] Means within the same row followed by different uppercase superscript letters differ $P \leq 0.01$ (**); $P \leq 0.001$ (***)
[1] Degrees of freedom: 3 for treatments at numerator; 20 per treatment for least square means at denominator
[2] Degrees of freedom: Model = 3; Error = 8; Total correct: 11
[3] Degrees of freedom: Model = 3; Error = 20; Total correct: 23
[4] Data processed through Kruskal-Wallis analysis
[5] Degrees of freedom: Model = 3; Error = 8; Total correct: 11
[6] Degrees of freedom: Model = 3; Error = 20; Total correct: 23

Slaughter Yields, Breast Yields, and pHu Values

At first slaughter at 12 day of age, CONTROL group birds had higher breast yield than VACC-LE plus birds (15.0 vs 13.0% slaughter weight –SW-, respectively; $P < 0.01$) despite their lower SW (Table 5.4). At second slaughter at 25 day of age, COX group chickens showed significantly higher breast yield than CONTROL group birds (19.6 vs 17.8% SW, respectively; $P < 0.01$) due to the higher breast weight of the former (249 vs. 224 g; $P < 0.01$). Although showing lower SW than CONTROL and COX ($P < 0.05$) birds, VACC group birds had intermediate breast weight and breast yield (18.6% SW) compared to the other 3 treatments. At commercial slaughter, breast yield was higher in animals treated with the coccidiosis control program (average 23.4%) than those in the CONTROL group (22.5% SW; $P < 0.001$). The values of cranial and caudal pHu (Table 5.4) measured on the breasts of birds slaughtered at 12 d of age were not affected by the experimental treatments. At 25 d of age, cranial pHu of VACC-LE plus birds was higher than in CONTROL birds (6.29 vs 6.16; $P < 0.05$), also COX and VACC birds (6.15 and 6.14, respectively; $P < 0.01$). At 51 d of age, statistical differences among groups emerged in both cranial and caudal regions. The pHu was significantly ($P < 0.01$) lower in breasts of CONTROL group birds than VACC birds measured at cranial level and those in VACC-LE plus group at caudal level. The significance of the covariate indicates a strong SW effect on breast weight and breast yield (% SW), and also on breast pHu measured in the caudal regions of birds sacrificed at 51 d of age. Analogously, breast yield (% carcass weight - CW-) was also influenced by SW (P COV < 0.001) and was also strongly affected by the treatments (Table 5.5). As regards the latter, CONTROL chickens had the lowest carcass and breast yields (75.0 and 30.0%, respectively) at commercial slaughter age. The carcass yield was in favor of birds treated with coccidiosis control programs (77.0 and 76.5% for COX and VACC-LE plus, respectively), whereas breast yield referred to CW was higher in VACC and VACC-LE groups than in the CONTROL group ($P < 0.05$) (Table 5.5). As observed by Kuttappan et al. (2013b) and Petracci et al. (2013a), it can be confirmed that heavier birds exhibit favorable breast yields. Animals treated with coccidiosis control programs showed better breast yields, even after covariate adjustment, thus suggesting a positive effect of the treatment regardless of SW. The feeding plan (VACC vs VACC-LE plus) did not reveal any remarkable or statistically significant differences in the yields considered, indicating that C3 and C4 diets fulfilled energy and nutrient requirements, even if with lower GE content. Lighter breasts were characterized by lower pH values; these findings are similar to those of previous studies by Petracci et al. (2013a,b), who observed lower pH values in standard yield hybrids compared to higher breast yield hybrids. Selection for increased breast muscle mass associated with hypertrophy can lower both glycolytic potential and activity by reducing glycolytic storage,

thus resulting in heavier breasts with higher pHu (Berri et al., 2004; Le Bihan-Duval et al., 2008).

Table 5.4 Slaughter and breast yields, and *Pectoralis major* muscle pH at 12, 25 and 51 d of age.

Attributes	Treatments (T)				SE	Probability	
	CONTROL	COX	VACC	VACC – LE plus		T	COV slaughter weight
Slaughter weight, SW (g)							
12 d[1, 3]	353[b]	405[ab]	393[ab]	423[a]	12	*	-
25 d[1, 3]	1335[a]	1298[a]	1157[b]	1262[ab]	28	*	-
51d[2, 4]	3489[b]	3667[a]	3593[ab]	3672[a]	36	**	-
Whole breast weight (g)							
12 d[1, 3]	58.8[A]	56.2[A]	55.2[AB]	51.2[B]	1.0	**	***
25 d[1, 3]	224[B]	249[A]	236[AB]	237[AB]	3.5	**	**
51 d[2, 4]	811[B]	837[A]	840[A]	845[A]	4.8	***	***
Breast yield (% SW)							
12 d[1, 3]	15.0[a]	14.3[a]	14.0[ab]	13.0[b]	0.3	**	**
25 d[1, 3]	17.8[B]	19.6[A]	18.6[AB]	18.7[AB]	0.3	**	*
51 d[2, 4]	22.5[B]	23.2[A]	23.3[A]	23.4[A]	0.1	***	***
Cranial pHu							
12 d[1, 3]	6.15	6.29	6.34	6.25	0.05	NS	NS
25 d[1, 3]	6.16[ABb]	6.15[B]	6.14[B]	6.29[Aa]	0.03	*	NS
51 d[2, 5]	5.96[B]	6.06[AB]	6.14[A]	6.07[AB]	0.02	**	NS
Caudal pHu							
12 d[1, 3]	6.18	6.17	6.34	6.19	0.04	NS	NS
25 d[1, 3]	6.16	6.20	6.14	6.22	0.03	NS	NS
51 d[2, 5]	5.83[b]	5.91[ab]	5.92[ab]	5.96[a]	0.03	**	***

[a, b] Means within the same row followed by different lowercase superscript letters differ $P \leq 0.05$ (*)
[A, B] Means within the same row followed by different uppercase superscript letters differ $P \leq 0.01$ (**); $P \leq 0.001$ (***)
[1] 20 animals per Treatment
[2] 133 (CONTROL), 132 (COX), 129 (VACC) and 134 (VACC-LE plus) animals
[3] Degrees of freedom: 4 (treatment effect = 3, LW effect = 1) at numerator; 8 (treatment effect), 67 (LW effect) at denominator
[4] Degrees of freedom: Model = 4 (treatment effect = 3, LW effect = 1); error = 523 (20 for treatment at denominator, 503 for LW effect at denominator); total correct = 527
[5] Degrees of freedom: Model = 4 (treatment effect = 3, LW effect = 1); error = 205 (20 for treatment at denominator, 185 for LW effect at denominator); total correct = 209

Table 5.5 Slaughter and breast yields at 51 d of age.

Attributes	Treatments (T)				SE	Probability	
	CONTROL	COX	VACC	VACC-LE plus		T	COV slaughter weight
Carcass weight, CW (g)[1, 2]	2700[C]	2770[A]	2737[B]	2753[AB]	6.7	***	***
Carcass yield (% SW)[1, 2]	75.0[C]	77.0[A]	76.0[B]	76.5[AB]	0.2	***	NS
Breast yield (% CW)[1, 2]	30.0[b]	30.2[ab]	30.6[a]	30.6[a]	0.2	*	***

[a, b] Means within the same row followed by different lowercase superscript letters differ $P \leq 0.05$ (*)
[A, B] Means within the same row followed by different uppercase superscript letters differ $P \leq 0.001$ (***)
[1] 133 (CONTROL), 132 (COX), 129 (VACC) and 134 (VACC-LE plus) animals
[2] Degrees of freedom: Model = 4 (treatment effect = 3, LW effect = 1); error = 523 (20 for treatment at denominator, 503 for LW effect at denominator); total correct = 527

White Striping prevalence

Breast lesions attributable to WS were not observed in chickens at 12 d of age in any of the 4 experimental groups. Lesions were detected at 25 d of age in all 4 experimental groups with a prevalence ranging from 5.4% (CONTROL and COX) to 29.4% (VACC) but the difference was not statistically significant (Table 5.6). When considering WS mean scores (Table 5.7) of the 4 treatments obtained at 12 and 25 d of age, the previous results were confirmed. At 51 d of age, the total WS prevalence was on average very high (95.1%) but showed no difference among treatments. As reported above, WS prevalence was macroscopically scored as level 0, level 1, and level 2. At 25 d of age, only level 1 was detected, with no differences among treatments, whereas at 51 d of age, WS severity was distributed according to 4% to level 0 (treatment effect: NS), 29.4% to level 1 (treatment effect: $P < 0.05$), and 66.6% to level 2 (treatment effect: $P < 0.01$) (Table 5.6). At commercial slaughter age, the prevalence of moderate WS (level 1) was higher in the breasts of CONTROL and VACC than COX group birds (36.8 and 33.5% vs 19.1%; $P < 0.05$). On the contrary, the prevalence of severe WS (level 2) was higher in COX than CONTROL and VACC groups (77.6 vs 62.6 and 61.6%, respectively; $P < 0.01$), and also higher than in VACC-LE plus birds (64.4%; $P < 0.05$). The significance of the covariate highlights a strong SW effect on WS prevalence at 51 d of age, but not earlier. Despite being covariated with SW, WS prevalence was influenced by the treatments at the commercial slaughter age (Table 5.6). The mean WS scores in Table 7 highlight the effect of treatment at 51 d of age, showing a greater lesion severity for COX than CONTROL and VACC groups (1.79 vs 1.50 and 1.58, respectively; $P < 0.001$), whereas group VACC-LE plus birds exhibited an intermediate degree of severity (1.63). The results obtained in this study differ from those in literature. In our study, total WS prevalence was on average very high (95.1%), and with a higher contribution from WS level 2 (66.6%) than WS level 1 (29.4%). Total WS prevalence was much higher than that observed by Lorenzi et al. (2014) on broilers in similar weight range (3.0 to-3.8 kg): with 45.2% at level 1 and 7.5% at level 2. Kuttappan et al. (2012a), Petracci et al. (2013a), Lorenzi et al. (2014) found that higher breast yield genotypes are more prone to develop WS lesions. Moreover, heavier fillets tend to exhibit a greater degree of severity. The significance of the covariate can confirm this relationship. Genetic selection for more favorable commercial traits has implications for both product quality and animal welfare, given that skeletal muscle development and metabolism are both altered and this leads to an elevated incidence of spontaneous and stress-induced myopathies. Higher muscle hypertrophy may not be supported by adequate capillary density and may lead to diminished oxygen and nutrient supply to the muscle and to lower catabolite removal, with undesirable consequences on meat quality, especially when animals are exposed to metabolic loads (Hoving-Bolink et al., 2000).

Regardless of SW or breast weight, 51-day-old COX group birds showed higher level 2 WS prevalence than VACC groups, assuming the use of ionophores against coccidiosis as a predisposing WS factor. This could be partially due to the protective effect of anticoccidials on intestinal integrity that leads birds to exhibit higher live performance in the first growing period but mainly to their proven toxicity on muscle fiber cells (Novilla, 1992; Chapman et al., 2010; Markiewicz et al., 2014). Moreover, Sandercock and Mitchell (2003) suggested that treatment with monensin could increase Na^{+} and Ca^{2+} intracellular concentration, resulting in increased Ca^{2+} accumulation (from outside and due to altered compartmentalization) and concomitant high CK loss. This is signal of tissue damage associated with degenerative myopathy, and its concentration, together with other enzymes, and is elevated in severe WS conditions. WS seems unrelated to systemic infectious or inflammatory conditions because birds with normal and seriously affected breasts did not show any difference in haematological parameters (Kuttappan et al., 2013b). The fact that VACC birds had the same WS prevalence level 2 and mean WS scores as CONTROL birds proves the absence of any vaccination effect on WS appearance. VACC-LE plus diets (C3 and C4) were tested with the intention of slowing down bird growth for the purpose of reducing the WS prevalence caused by rapid growth, as was recently observed by Kuttappan et al. (2012a), who also fed birds with high and low energy diets. As the chickens in our study utilized nutrients with the diets C3 and C4 more efficiently, due to higher amino acids and enzymes supplementation, final slaughter weight was comparable to that of the VACC group. Unfortunately, the likely higher nutrient bioavailability at muscle tissue level was not able to restrain WS prevalence. In conclusion, this study has demonstrated that both of the coccidiosis control programs adopted were capable of increasing growth and carcass yields over those of untreated chickens. It has also shown that the early (25 d of age) macroscopic appearance of WS was not affected by the experimental treatments. At commercial slaughter age, total average WS prevalence was 96%, with a level 2 prevalence that was significantly higher for COX birds, even though SW had been included as covariate. This study suggests that vaccination against coccidiosis has no effect on WS occurrence.

Table 5.6 White Striping prevalence observed on Pectoralis major muscle at 12, 25, and 51 d of age.

	Treatments (T)				SE	Probability	
Prevalence	CONTROL	COX	VACC	VACC – LE plus		T	COV slaughter weight
Total prevalence							
12 d[1, 3]	0	0	0	0	-	-	-
25 d[1, 3]	5.4	5.4	29.4	5.8	8.2	NS	NS
51d[2, 4]	98.4	96.7	96.1	92.7	1.6	NS	*
Level 0 prevalence (absence)							
12 d[1, 3]	100	100	100	100	-	-	-
25 d[1, 3]	94.6	94.6	70.6	94.2	8.2	NS	NS
51d[2, 4]	1.6	3.3	3.9	7.3	1.6	NS	*
Level 1 prevalence							
12 d[1, 3]	0	0	0	0	-	-	-
25 d[1, 3]	5.4	5.4	29.4	5.8	8.2	NS	NS
51d[2, 4]	36.8[a]	19.1[b]	33.5[a]	28.3[ab]	3.4	*	*
Level 2 prevalence							
12 d[1, 3]	0	0	0	0	-	-	-
25 d[1, 3]	0	0	0	0	-	-	-
51 d[2, 4]	61.6[B]	77.6[Aa]	62.6[B]	64.4[ABb]	2.8	**	***

[a, b] Means within the same row followed by different lowercase superscript letters differ $P \leq 0.05$ (*)
[A, B] Means within the same row followed by different uppercase superscript letters differ $P \leq 0.01$ (**); $P \leq 0.001$ (***)
[1] 20 animals per Treatment
[2] 133 (CONTROL), 132 (COX), 129 (VACC) and 134 (VACC-LE plus) animals
[3] Degrees of freedom: Model = 4 (Treatment effect = 3, LW effect = 1); Error = 7; Total correct: 11
[4] Degrees of freedom: Model = 4 (Treatment effect = 3, LW effect = 1); Error = 19; Total correct: 23

Table 5.7 Non-parametric Kruskal-Wallis test on the mean WS scores on *Pectoralis major* muscle at 12, 25 and 51 d of age.

	Treatments (T)				P
Mean score[1]	CONTROL	COX	VACC	VACC-LE plus	
12 d[2]	0	0	0	0	NS
25 d[2]	0.15 ± 0.37	0.10 ± 0.31	0.15 ± 0.37	0.05 ± 0.22	NS
51d[3]	1.50 ± 0.57[B]	1.79 ± 0.46[A]	1.58 ± 0.57[B]	1.63 ± 0.60[AB]	***

[a, b] Means within the same row followed by different lowercase superscript letters differ $P \leq 0.05$ (*)
[A, B] Means within the same row followed by different uppercase superscript letters differ $P \leq 0.001$ (***)
[1] Degrees of freedom: 3
[2] 20 animals per Treatment
[3] 133 (CONTROL), 132 (COX), 129 (VACC) and 134 (VACC-LE plus) animals

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CHAPTER 6

Relationship between hardness and myowater properties in Wooden Breast affected chicken meat: a nuclear magnetic resonance study.

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ABSTRACT

The role of myowater-holding on the development of the hardness of Wooden Breast (WB) affected chicken breasts was investigated. Transverse (T_2) relaxation times and proportions of myowater populations (T_{2B}, T_{21} and T_{22}) were assessed using low-field nuclear magnetic resonance (NMR) relaxometry and integrated with meat compression measurements. Two muscle conditions (M: Normal (N) vs WB), four sampling locations (L), four sampling times (T) and interactions (M x L and M x T) were considered. Compared to N, WB was harder, the extramyofibrillar myowater population (T_{22}) was increased and the relaxation time of the water trapped into the myofibrillar matrix (T_{21}) was also increased. A link between the T_{21} relaxation time of water trapped into the myofibrillar matrix and hardness was suggested for the WB muscles. During storage, a redistribution of water occurred over time, as revealed by an increasing trend of the T_{21} population, but a concomitant texture evolution did not reflect this change. The cranial/superficial part of the breasts exhibited the highest amount of the extramyofibrillar water population (T_{22}), and the texture of this muscle part was harder than the deep layers. However, the role of myowater on muscle hardness was not fully clarified by this study.

Keywords: broiler, woody breast, NMR relaxation times, water populations**,** texture

1. Introduction

An emergent myopathy in fast growing, meat-type broiler chickens has been described and named Wooden Breast (WB) (Sihvo, Immonen, & Puolanne, 2014). Macroscopically, the affected *Pectoralis major* muscle is hard, pale, outbulging and sometimes superficially covered with small haemorrhages, exudate and occasionally White Striping; extended areas with poor cohesion of the muscle bundles are also visible beneath the lesioned areas (Dalle Zotte et al., 2017, Chapter 3). Histologically, the condition was defined as a moderate or severe polyphasic myodegeneration with regeneration of the muscle tissue; therefore, inflammation and necrosis are detected and are followed by accumulation of a variable amount of interstitial connective tissue (fibrosis) as reparative response (Sihvo et al., 2014). Starting from the first surveys on WB and until more recent studies, the fibrotic response has been considered as the primary factor for the typical hardness of the affected tissue (Sihvo et al., 2014, Clark & Velleman, 2016), as extensive hard consistency of raw meat and increased intramuscular collagen often occur together (Sihvo et al., 2014; Petracci et al., 2015; Soglia et al., 2016a; Chatterjee, Zhuang, Bowker, Rincon, & Sanchez-Brambila, 2016). Interestingly, it was demonstrated that WB muscle does not possess a homogeneous structure, as fibrosis was found to affect the anterior portions of the fillets, whereas the middle-ventral and postero-ventral locations were less or not at all affected (Clark & Velleman, 2016). In addition, contrary to normal breast muscles, also superficial and deep layers in raw affected *Pectoralis major* muscle has been found to differ in terms of textural properties, which probably reflects a variation in the muscle architecture between layers. Indeed, according to Gao (2015), hard consistency of the muscle is mainly present at the surface level at the early *post mortem* stage and becomes as soft as the normal condition during a chilled storage. As previously mentioned, fibrosis has been considered the major reason for the hard texture of affected chicken breasts so far. However, some cases of hard breasts without a significant accumulation of collagen were detected in broiler chickens both in very young birds (Sihvo, Lindén, Airas, Immonen, & Puolanne, 2017a; Sihvo et al., 2017b) and at commercial slaughter age (Sihvo et al., 2014; Dalle Zotte et al., 2017, Chapter 3), thus opening new hypotheses on the reasons for muscle hardness development. One hypothesis may involve the role of myowater. Indeed, changes in muscle microstructure directly affect water distribution among the three water populations defined by nuclear magnetic resonance (NMR) T_2 relaxation studies: T_{2B} (H_2O closely associated to macromolecules/proteins), T_{21} (H_2O trapped into myofibrillar matrix) and T_{22} (extramyofibrillar H_2O), with each water compartment exhibiting its typical relaxation time (Bertram et al., 2001; Bertram, Purslow, & Andersen, 2002). In a previous study of Soglia, Laghi, Canonico, Cavani, & Petracci (2016b), the WB condition resulted in a remarkable decrease in the intramyofibrillar fraction and a

concomitant increase in the extramyofibrillar water fraction. Consequently, the present study aimed to investigate the role of the chemical-physical state of myowater on hardness in WB affected chicken meat, integrating low-field NMR relaxometry with texture analysis (compression test). NMR properties and hardness were evaluated not only according to muscle condition but also considering a 72 h *post mortem* chilled storage and four different sampling locations, as these factors have been shown to affect meat textural properties (Soglia et al., 2017).

2. Material and methods

Samples collection and preparation

During two different sampling times, a total of 96 breast muscles from 34-day-old broiler chickens were collected at a commercial Danish slaughterhouse (Danpo A/S, Aars, Denmark). Each time, 48 fillets were selected according to the presence or the absence of severe Wooden Breast lesions, thus obtaining 24 macroscopically unaffected or normal (N) and 24 Wooden Breast (WB) samples. The selection based on the visual and palpatory inspection of *Pectoralis major* muscles; breasts exhibiting diffused hardened areas were scored as WB. The presence of bulges, pale colour and the surface covered with exudate, haemorrhages and white striping were also detected in the selected WB breasts (Dalle Zotte et al., 2017, Chapter 3; Sihvo et al., 2014). On the contrary, fillets with soft and elastic tissue with uniform colour were scored as N. After selection, breasts were immediately packed into polyethylene bags, kept cool and transported to the NMR laboratory of the Department of Food Science (Aarhus University, Årslev), where further analyses took place. At the laboratory, fillets were kept at 4 °C for four different storage times: 10, 24, 48 and 72 hours *post mortem* (*pm*); 6 WB-affected (WB) and 6 normal (N) fillets were used for each time. Four stripes per breast (1 cm x 1 cm x 4 cm, 5 ± 0.5 g) were excised parallel to the fibre direction; two of them were obtained from the cranial end of the fillet (CRA), whereas the other two were cut from the medial portion (MED). Within each portion, one stripe was snipped from the superficial layer (S: 0.2-1.2 cm deep under the muscle surface) and the other was snipped from the deep layer (D: 1.5-2.5 cm deep under the muscle surface) (Gao, 2015; Soglia et al., 2017). Locations were named as follows: CRA/S = cranial/surface; CRA/D = cranial/depth; MED/S = medial/surface; MED/D = medial/depth (Figure 6.1). Accordingly, 24+24 meat samples were prepared and analysed per each of the eight measurement days.

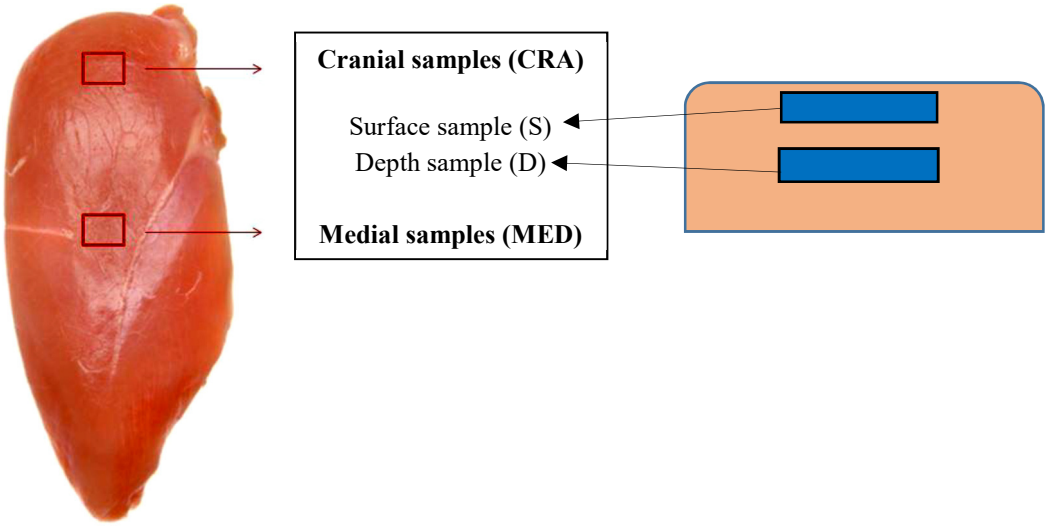

Figure 6.1 Sampling locations.

NMR measurements

Meat stripes (1 cm x 1 cm x 4 cm) were placed in glass test tubes, which were sealed with paraffin film and thermostated at 25 °C for 20 min in a waterbath. Thereafter, transverse relaxation time (T_2) measurements were performed on a Maran Benchtop Pulsed NMR Analyser (Resonance Instruments Ltd, Witney, UK) equipped with a 18 mm probe head operating at a magnetic field strength of 0.47 T and a corresponding resonance frequency for protons of 23.2 MHz. Transverse relaxation time (T_2) was measured using the Carr-Purcell-Meiboom-Gill (CPMG) spin-echo sequence (Carr & Purcell, 1954; Meiboom, Gill, Huxley, & Niedergerke, 1958) and a τ-value (time between 90 and 180° pulse) of 150 µs. Data from 4096 echoes were acquired as 16 scan repetitions. The obtained T_2 data were analyzed using distributed exponential fitting analysis according to the regularization algorithm by Butler, Reeds, & Dawson (1981) and carried out in MatLab (The Mathworks Inc., Natick, MA, USA) using in-house scripts. Plots of relaxation amplitudes for individual relaxation processes vs relaxation times revealed the presence of three relaxation populations. For each of the three water populations T_{2B}, T_{21} and T_{22}, relaxation times were calculated from the peak position and proportions of protons exhibiting those relaxation times were calculated from the corresponding area under each peak, using an in-house programme written in Mathlab (The Mathworks Inc., Natick, MA, USA).

Hardness measurements

After the NMR analyses, the same meat samples were then subjected to a single compression test in order to determine their hardness. A Brookfield CT3 texturometer (Texture Technologies Co, Hamilton, MA, USA) equipped with a 250 N load cell was used, setting the trigger load at 0.3 N and the

test speed of at 50 mm/min (0.83 mm/s). One sample from each muscle (48) was taken at each storage time (10, 24, 48 and 72 h *pm*). Strips were cut before analysis (1 cm x 1 cm x 3 cm) in order to fit to the compression probe (1 cm^2) and to the measuring cell (1 cm x 1 cm x 3 cm), which were modified according to Lepetit & Culioli (1994) and Campo, Sañudo, Panea, Alberti, & Santolaria (1999). Strips were compressed to 80% of their initial height (Lyon & Lyon, 2001) perpendicularly to the fibre direction, which, therefore, could extend only longitudinally. From each measurement, a curve was obtained and the peak force represented the maximum hardness (expressed in newtons).

Statistical analysis

The "Location" (L) effect was created combining breast portion (CRA/MED) and layer (S/D); four locations resulted from the combinations (CRA/S; CRA/D; MED/S; MED/D). Then, data were analysed using a SAS 9.1.3 statistical software package for Windows (SAS, 2004). Variables were evaluated by ANOVA, choosing a mixed model (PROC MIXED) which considered muscle condition (M: N; WB), location (L) , hour *pm* (T: 10h, 24h, 48h, 72h) and sampling time (1; 2) as fixed effects, whereas sample (breast) was considered as random repeated effect. The interactions M x T and M x L were also studied. Post-hoc pairwise comparisons were evaluated by Bonferroni adjustments. Pearson correlations between hardness and NMR variables were performed with the two muscle conditions separately considered. Two significance levels were assigned: $P \leq 0.05$ and $P \leq 0.01$.

3. Results and discussion

The present study investigated the role of the physico-chemical state of myowater on the development of hardness in Wooden Breast affected chicken breast muscles by NMR relaxometry and texture analysis (compression test). The considered variables were affected by muscle condition (M), storage time (T), sampling location (L) and their interaction (M x T and M x L). In Table 6.1, the overall effects of the main variable (muscle condition; WB or N) on NMR relaxation times and hardness across all other variables are presented. WB breasts were characterized by longer T_{2B} and T_{21} relaxation times than N breasts (T_{2B}: 0.82 vs 0.50 ms; T_{21}: 21.0 vs 13.6 ms; $P < 0.01$). The proportions of T_{2B} (the water closely associated to macromolecules) and T_{21} (water trapped into the myofibrillar matrix) populations were lower in WB than in N samples (T_{2B}: 4.47 vs 5.29 %; T_{21}: 88.5 vs 93.4 %, $P < 0.01$) in favour of extramyofibrillar water, T_{22} population (7.05 vs 1.26 %, $P < 0.01$). WB breasts clearly experienced changes in muscle structure, and structural differences affect water mobility and distribution as detected by NMR T_2 relaxometry (Bertram et al., 2002). This can be ascribed to the fact that T_2 relaxation will reflect water-protein interactions and thereby also depend on spatial factors

as these will influence the probability of a water molecule to meet a protein acting as a relaxation sink (Hills, Takacs, & Belton, 1990). In association with this hypothesis, it has been shown that a close relationship exists between meat structure and T_2 relaxation rate of water populations in meat (Bertram et al., 2002). Furthermore, structural features of intrinsic meat proteins can also influence T_2 relaxation through their ability to act as relaxation sinks. Thus, conformation changes in protein structures caused by pH variations and/or changes in exposure of hydrophobic groups are also reflected in the NMR T_2 relaxation pattern of meat (Bertram, Whittaker, Andersen, & Karlsson, 2003). Interestingly, sarcomere length as well as the tensile strength were found to increase in WB-affected breasts (Ababei, 2016; Tijare et al., 2016). Therefore, muscle tactile stiffness and contracted appearance in severe WB cases could not be related to a general sarcomere shortening. On the contrary, according to Gordon, Huxley, & Julian (1966), sarcomere stretching seemed to be related to an increase in muscular tension, and their relationship was described by a bell-shaped curve. At the same time, sarcomere length has been demonstrated to be highly correlated (r = 0.84) to the T_{21} relaxation time constant (Bertram et al., 2002), thus indicating that structural changes involving sarcomere length also affect the behaviour of this water population. Therefore, the present results suggest a connection relating muscle structure, the T_2 relaxation time of water trapped into the myofibrillar matrix and muscle hardness.

Table 6.1 Effect of the two muscle conditions (M: Wooden Breast, WB; Normal, N) on NMR T_2 relaxation times and populations percentages, and hardness. Total number of samples: 96 (48 WB and 48 N muscles, collected during two samplings). The table shows mean values of all breasts, which were analyzed at 10, 24, 48 and 72 h *pm* and sampled at the level of the four locations (CRA/S, CRA/D, MED/S, MED/D).

Variables	Muscle condition (M)		*P*-value
	WB	N	
No. of breasts	48	48	
Time constants (ms)			
T_{2B}	0.82 ± 0.03 ^{A}	0.50 ± 0.03 ^{B}	<0.01
T_{21}	21.0 ± 0.5 ^{A}	13.6 ± 0.5 ^{B}	<0.01
T_{22}	59.4 ± 3.1	65.7 ± 3.1	0.1608
Populations (%)			
T_{2B}	4.47 ± 0.07 ^{B}	5.29 ± 0.07 ^{A}	<0.01
T_{21}	88.5 ± 0.3 ^{B}	93.4 ± 0.3 ^{A}	<0.01
T_{22}	7.05 ± 0.28 ^{A}	1.26 ± 0.28 ^{B}	<0.01
Hardness			
Compression (N)	24.8 ± 0.6 ^{a}	23.0 ± 0.6 ^{b}	<0.05

$^{a, b}$ Means within the same row followed by different lowercase superscript letters differ $P \leq 0.05$;
$^{A, B}$ Means within the same row followed by different uppercase superscript letters differ $P \leq 0.01$.

The correlation between compression and NMR relaxation times was studied within the two muscle conditions (Table 6.2). Interestingly, hardness was significantly ($P < 0.01$) correlated with T_{2B} ($r = 0.48$), T_{21} ($r = 0.46$) and T_{22} ($r = 0.41$) relaxation times only in the WB group. The analysis of correlation between texture and NMR relaxation times agreed with the statement of Pearce et al. (2008). They investigated the relationship occurring between shear force and NMR relaxation times in lamb *M. longissimus dorsi* during a storage period, and they found a relationship of $r = 0.78$ between NMR relaxation measurements and shear force. The data also suggest a positive correlation between the T_{21} relaxation time and the compression force, but only in WB meat.

Table 6.2 Correlations between NMR parameters (relaxation times and populations percentages) and hardness, evaluated considering the two muscle conditions (M: Wooden Breast, WB; Normal, N) separately. Total number of samples: 96 (48 WB and 48 N muscles, collected during two samplings). The table considers mean values of breasts analyzed at 10, 24, 48 and 72 h *pm,* and sampled at the level of the four locations (CRA/S, CRA/D, MED/S, MED/D).

Muscle condition	WB		N	
Variables	Compression (N)	*P*-value	Compression (N)	*P*-value
Time constants (ms)				
T_{2B}	0.48	<0.01	0.15	<0.05
T_{21}	0.46	<0.01	0.13	0.0891
T_{22}	0.41	<0.01	-0.02	0.8489
Populations (%)				
T_{2B}	-0.06	0.4093	-0.22	<0.01
T_{21}	-0.21	<0.01	0.02	0.7579
T_{22}	0.21	<0.01	0.18	<0.05

Correlations ≥ 0.40 were considered high and significant when $P \leq 0.05$.

Table 6.3 shows the effects of storage time on NMR relaxation times, water population percentages and muscle hardness. Hardness was not influenced by the interaction M x T ($P > 0.05$), similarly to Soglia et al. (2017), although a weak tendency for an aging-type tenderisation may be seen in both studies. The T_{21} relaxation times were generally longer in WB group than in N group, and the times did not change during the storage period within the groups. The proportion of the population T_{2B} was not affected by the interaction M x T ($P > 0.05$), but for the T_{21} the overall M x T effect was significant ($P < 0.040$). The T_{21} population was in general higher in N samples than in the WB samples ($P = 0.01$), and for the N samples this water population remained stable during storage, while in the WB samples the values were higher in the 72h samples than in the 10h samples ($P = 0.01$). The T_{21}population was significantly higher in WB samples than the N ones throughout the storage peri-

od ($P = 0.01$), with the exception of WB 72h, which was statistically similar to N 10h. In addition, T_{22} percentages of WB samples decreased from 10 to 72 hours *post mortem*, whereas the same fraction, being also lower than in WB, remained unchanged throughout the cold storage period in N samples ($P = 0.01$). Straadt, Rasmussen, Andersen, & Bertram (2007) and Pearce et al. (2008) indicated for pork and sheep meats respectively, a tenderising action of storage in association with a redistribution of water that was shown as a lower amount of the T_{22} population in favour of the intramyofibrillar compartment T_{21}. The observations of the current study on chicken meat did not agree with the observations collected for other meat species. Indeed, differently from the cited studies, normal breast meat did not experience a change in the water distribution during ageing ($P > 0.05$); on the contrary, storage time affected the T_{21} population percentage and that of the T_{22} population in WB breasts. At the same time, an expected association between changes in water compartmentalisation and a more tender meat was not corroborated by the compression results. Indeed, samples analysed at different times and/or characterised by different muscle conditions exhibited a similar hardness (Table 6.3). The effect of the interaction between muscle condition and sampling location (M x L) on myowater NMR relaxation times, myowater populations percentages and breast meat hardness is shown in Table 6.4. Overall, the study of the interaction M x L revealed that the NMR relaxation properties of myowater markedly differed between the two conditions. In WB affected samples, the Location CRA/S possessed the longest T_{2B} and T_{21} relaxation times, contrary to all the other locations. All the four sampling locations of normal breasts had similar T_{2B} and T_{21} relaxation times. The two groups exhibited rather similar T_{22} relaxation times, except for the significant differences ($P < 0.01$) observed between N MED/D vs N CRA/S, WB CRA/D and WB MED/D. The overall interaction M x L did not affect the proportion of the T_{2B} population, but on the contrary, the T_{21} and T_{22} populations were significantly influenced. In detail, all the four locations of normal samples had similar T_{21} percentages, which were higher in particular than that of Location CRA/S of WB samples ($P < 0.01$). On the contrary, in WB group, Location CRA/S had the highest T_{22} population, followed by MED/S and then CRA/D and MED/D together ($P = 0.01$). Again, concerning T_{22} population percentage, all N Locations had similar and lower values than those of WB ($P = 0.01$). Globally, Location CRA/S was harder than Locations CRA/D and MED/D, while the differences between CRA/S and MED/S both in WB and N groups were not significant ($P > 0.01$). Contrary to the expectations based on the study of Soglia et al. (2017), the CRA/S layers of WB and N breasts exhibited the same hardness values. pH is known to affect the water-holding capacity (Hamm, 1972; Puolanne & Halonen, 2010) as well as firmness of raw meat, although there are not published papers on that subject. The pH difference observed between WB and N breast muscles has been about 0.1-0.2 units (Soglia et al., 2016a), and the value is around 6.0 in modern fast-growing birds,

which would mean in other meat animals so called dark cutting meat, but with WB this is not relevant. At this level pH means higher water-holding (Hamm, 1972), which is the contrary to what WB causes in meat. Higher pH means also increased firmness in raw meat like dark-firm-dry beef, but not even close to the extent that would be comparable to the WB hardness of focal WB of young birds, and especially not of diffuse cases of WB (Sihvo et al. 2014, Sihvo et. al., 2017b).

4. Conclusions

This study demonstrated that the Wooden Breast condition in meat-type broiler chickens is associated with a different water distribution and myowater properties compared with muscles without Wooden Breast lesion, as indicated by the increased T_2 relaxation times for myofibrillar water and partial water relocalisation to extramyofibrillar spaces in Wooden Breast samples. A connection relating increased muscle hardness and longer relaxation time of water trapped into the myofibrillar matrix was also found; however, an association between changes in water NMR relaxation times and meat hardness was found only for the WB condition and not in Normal meat.

Table 6.3 Effect of the interaction between muscle condition (M) and Time (T) on NMR T_2 relaxation times and population percentages, and hardness. Total number of samples: 96 (48 WB and 48 N muscles, collected during two samplings). Values of each time consider all the four locations together (CRA/S, CRA/D, MED/S, MED/D).

Muscle condition (M)	WB				N				*P*-value
Hours *p.m.* (T)	10	24	48	72	10	24	48	72	M x T
Variables									
No. of breasts	12	12	12	12	12	12	12	12	
Time constants (ms)									
T_{2B}	0.76 ± 0.06 ^{ABC}	0.84 ± 0.06 ^{AB}	0.67 ± 0.06 ^{BCD}	1.00 ± 0.06 ^{A}	0.49 ± 0.06^{CD}	0.46 ± 0.06^{D}	0.56 ± 0.06^{BCD}	0.49 ± 0.06^{CD}	<0.01
T_{21}	20.2 ± 1.0 ^{A}	22.5 ± 1.0^{A}	18.8 ± 1.0 ^{AB}	22.5 ± 1.0 ^{A}	13.3 ± 1.0^{C}	12.6 ± 1.01 ^{C}	14.6 ± 1.0^{BC}	13.9 ± 1.0 ^{BC}	<0.05
T_{22}	59.2 ± 6.1	68.6 ± 6.2	49.4 ± 6.2	60.6 ± 6.3	65.0 ± 6.2	77.6 ± 6.3	69.4 ± 6.4	50.6 ± 6.0	0.1236
Populations (%)									
T_{2B}	4.63 ± 0.14	4.48 ± 0.14	4.45 ± 0.14	4.31 ± 0.15	5.49 ± 0.14	5.59 ± 0.14	5.05 ± 0.14	5.04 ± 0.13	0.3251
T_{21}	86.6 ± 0.5 ^{D}	87.8 ± 0.5 ^{CD}	89.3 ± 0.5 ^{CD}	90.1 ± 0.5 ^{BC}	92.9 ± 0.5 ^{AB}	93.6 ± 0.5 ^{A}	93.7 ± 0.5^{A}	93.5 ± 0.5 ^{A}	<0.05
T_{22}	8.73 ± 0.56 ^{A}	7.66 ± 0.56 ^{AB}	6.22 ± 0.56 ^{AB}	5.57 ± 0.58 ^{B}	1.62 ± 0.6^{C}	0.79 ± 0.6 ^{C}	1.15 ± 0.6 ^{C}	1.48 ± 0.5 ^{C}	<0.05
Hardness									
Compression (N)	26.6 ± 1.2	26.4 ± 1.2	21.9 ± 1.2	24.4 ± 1.2	24.4 ± 1.2	22.2 ± 1.2	24.0 ± 1.2	21.3 ± 1.2	0.0572

$^{A, B}$ Means within the same row followed by different uppercase superscript letters differ $P \leq 0.01$.

Table 6.4 Effect of the interaction between muscle condition (M) and Location (L) on NMR T_2 relaxation times and population percentages, and hardness. Total number of breasts: 96 (48 WB and 48 N muscles, collected during two samplings). The mean values of each location (CRA/S, CRA/D, MED/S and MED/D), consist in the mean of values obtained at 10, 24, 48 and 72 h *pm.*

Muscle condition (M)	WB				N				*P*-value
Locations (L) Variables	CRA/S	CRA/D	MED/S	MED/D	CRA/S	CRA/D	MED/S	MED/D	M x L
No. of samples	48	48	48	48	48	48	48	48	
Time constants (ms)									
T_{2B}	1.23 ± 0.05 ^{A}	0.63 ± 0.04 ^{C}	0.81 ± 0.05 ^{B}	0.60 ± 0.04 ^{C}	0.51 ± 0.04^{C}	0.50 ± 0.04 ^{C}	0.51 ± 0.04 ^{C}	0.49 ± 0.04 ^{C}	< 0.01
T_{21}	26.1 ± 0.7 ^{A}	17.9 ± 0.6 ^{C}	23.0 ± 0.6 ^{B}	17.0 ± 0.6 ^{C}	14.1 ± 0.62 ^{DE}	13.1 ± 0.6^{E}	13.8 ± 0.6 ^{E}	13.3 ± 0.6 ^{E}	< 0.01
T_{22}	69.8 ± 5.2 ^{AB}	50.1 ± 4.6 ^{B}	66.2 ± 5.0 ^{AB}	51.6 ± 4.6 ^{B}	52.5 ± 4.7 ^{B}	67.7 ± 4.8 ^{AB}	63.5 ± 4.7 ^{AB}	79.0 ± 5.0 ^{A}	< 0.01
Populations (%)									
T_{2B}	4.11 ± 0.14	4.79 ± 0.12	4.07 ± 0.13	4.92 ± 0.12	4.95 ± 0.12	5.58 ± 0.13	5.09 ± 0.12	5.55 ± 0.12	0.4123
T_{21}	85.0 ± 0.4 ^{D}	90.8 ± 0.4 ^{B}	87.6 ± 0.4 ^{C}	90.6 ± 0.4 ^{B}	93.5 ± 0.4^{A}	93.3 ± 0.4 ^{A}	93.6 ± 0.4 ^{A}	93.3 ± 0.4 ^{A}	< 0.01
T_{22}	10.9 ± 0.5 ^{A}	4.42 ± 0.41 ^{C}	8.37 ± 0.44 ^{B}	4.47 ± 0.42 ^{C}	1.57 ± 0.42^{D}	1.10 ± 0.43 ^{D}	1.25 ± 0.42 ^{D}	1.12 ± 0.43 ^{D}	< 0.01
Hardness									
Compression (N)	30.9 ± 1.0 ^{A}	20.6 ± 0.9 ^{C}	26.3 ± 1.0 ^{AB}	21.4 ± 0.9 ^{C}	26.3 ± 0.9 ^{AB}	21.0 ± 0.9 ^{C}	23.2 ± 0.9 ^{BC}	21.4 ± 0.9 ^{C}	<0.01

Locations: CRA/S = cranial/surface; CRA/D = cranial/depth; MED/S = medial/surface; MED/D = medial/depth.
$^{A, B}$ Means within the same row followed by different uppercase superscript letters differ $P \leq 0.01$.

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CHAPTER 7

General conclusions

Pursuing the pre-established goals, the studies conducted during these three-year PhD contributed in implementing the knowledge on these emerging myopathies. Specifically:

- The association between Wooden Breast (WB) and fillets morphometric traits (increased breast weight and thickness) was confirmed. Wooden Breast macroscopic "descriptors" were typified (presence of bulges, petechiae, fluid and clear exudate covering the cranial end of the muscle surface) and quantified; the histological traits of lesioned breasts evidenced the presence of fibres with greater size and a higher incidence of giant fibres. The technological evaluations highlighted that frozen storage did not exert an ameliorative effect on WB fillets pH, colour traits and water holding capacity; however, together with extensive poor cohesion of muscle tissue, frozen storage might have mitigated the expected hard texture.
- The mineral content profiles confirmed the hypothesis of a defective ions regulation occurring in White Striping (WS) and WB myopathies and triggering tissue damage. Moreover, it emerged that the simultaneous presence of WS and WB within the same fillet exerted an additional detrimental effect on either technological traits (pH, colour values, shear force and water holding capacity), nutritional quality, and toughness perception respect to the single White Striping lesions individually considered.
- Testing the impact of the coccidiosis prophylaxis methods, it emerged that vaccine and anticoccidial drug both improved live performances when compared to the untreated group. As total WS incidences were high in all groups at slaughter, it can be inferred that vaccination against coccidiosis did not exert a protective effect towards WS occurrence and severity. As the highest WS severity was detected in birds treated with the coccidiostat, it is likely that a synergistic action between higher slaughter weight and a possible ionophores toxicity on muscle fibres had occurred in this group. From the study, it was observed that low energy diet did not impair overall live performances when supplemented with aminoacids and enzymes. Moreover, the study contributed in outlining the age of WS onset, as the macroscopic white striations were detected already at 25 days of age irrespective from coccidiosis prophylaxis and diet energetic content.
- As pectoral muscles experience structural changes under WB conditions that are due to tissue degenerative changes, lesioned fillets also possess altered myowater distribution and properties compared to the unaffected counterparts. In detail, as indicated by NMR T_2 relaxometry studies, a partial water molecules relocalisation toward extramyofibrillar spaces occur in WB samples,

along with a higher mobility of water trapped into the myofibrillar matrix. A positive correlation relating increased muscle hardness and the greater mobility of water trapped into the myofibrillar matrix was discovered in WB fillets, thus opening a new hypothesis, alternative to fibrosis, to explain the characterising hard consistency of WB meat.

To help the poultry sector face these challenges and reduce the serious economic losses caused by WS and WB, the scientific community must find proper solutions to reduce the incidence of these defects; in the meantime, determining the usability of affected meat in further processed products is also urgent. Specifically:

- The role of broiler chickens genotype on the occurrence of WS and WB is not clear yet; moreover, it emerged from previous literature and from our studies, that these conditions are only partly influenced by environmental and management factors. Thus, a possible underlying genetic fingerprint of WS and WB has to be investigated in the future. Specifically, to reduce their occurrence, further research should be addressed towards the identification of the causative gene or group of genes in the different chicken hybrids. The second step should then consist in the development of hybrid selection programs, which avoid the transmission of the responsible genes and include birds' welfare among their goals.
- Affected chicken breasts are downgraded or discarded by the consumer at purchase. Consequently, impacted meat has firstly to be sorted at the slaughterhouse. The modern poultry industry requires to quickly remove the affected fillets from the production chain. Therefore, feasible and reliable, non-invasive on-line methods have to be developed to automatically sort affected from normal breasts, and then to grade WS and WB fillets according to the conditions severity. Interestingly, affected breasts could be rapidly discriminated on-line by spectroscopic techniques according to their proximate composition, or by spectrophotometric methods according to the muscle structure. Afterwards, affected meat has to be destined to further processing (trimming, grinding, etc.) and to manufacture meat products (nuggets, frankfurters, sausages, etc.). However, from previous literature and from our studies, it emerged that tissue degeneration occurring in WS and WB breast muscles, has detrimental effects on meat nutritive value, protein functionality (and thus on meat WHC and emulsifying properties) and texture, thus making affected meat processing challenging. Consequently, future research should assess the most appropriate levels of inclusion of WS and WB breast meat, which does not lead to an unacceptable qualitative decline of processed products. On one hand, these amounts might depend from the myopathies severity degrees; on the other hand, they might depend on the characteristics of the meat product (finer or coarser texture, addition of NaCl and other ingredients in the batter formulation, etc.). At exper-

imental level, several types of products (patties, nuggets, frankfurter, sausages, etc.) including affected breasts at different percentages should be formulated. Their rheological and textural characteristics, their nutritional and microbiological quality should be evaluated. Afterwards, assessing the consumer's preference and acceptability toward these products will be a key aspect to test.

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