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STRATEGIES FOR CONTROLLING MYOPATHIES AND CHARACTERIZATION OF MEAT QUALITY IN BROILER CHICKENS

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RIASSUNTO

Il settore avicolo si distingue dagli altri settori della produzione animale per le possibilità di crescita a livello mondiale legate alla maggiore richiesta di proteina animale da parte del consumatore e alle caratteristiche qualitative della carne avicola. Tuttavia, la selezione di animali caratterizzati da elevate velocità di crescita e elevate rese in petto, taglio principale, hanno accentuate la comparsa di difetti della qualità della carne, anche indicati come miopatie. Queste alterazioni sono identificabili prevalentemente in white striping e wooden breast, non rappresentano un problema per la salute umana, ma piuttosto un danno per il produttore e una perdita economica per il produttore. Si tratta infatti di alterazioni che modificano le proprietà sensoriali, nutrizionali e tecnologiche della carne e che possono pregiudicare la sua destinazione alla vendita diretta, comportare la sua utilizzazione per prodotti trasformati o, nei casi di alterazioni più severe, la distruzione e la perdita del prodotto. Sebbene la ricerca abbia dedicato molta attenzione al problema negli ultimi 5 anni, le conoscenze sull'eziologia delle miopatie e, soprattutto, sui fattori in grado di modificare la frequenza e il grado di queste alterazioni richiedono ulteriori approfondimenti.

Pertanto, la presente Tesi di dottorato ha inteso: *i*) valutare gli effetti di alcune strategie alimentari, restrizione alimentare di tipo quantitativo (precoce e tardivo) e gestione del fotoperiodo, sulle prestazioni produttive, sull'incidenza e il grado delle miopatie e sulla qualità della carne del pollo da carne; *ii*) studiare l'effetto del sesso sulle miopatie e la qualità del prodotto; *iii*) verificare la risposta dei diversi tipi genetici disponibili a livello commerciale; *iv*) approfondire come e quanto queste alterazioni influiscano sui principali aspetti di qualità della carne sul prodotto fresco e durante la conservazione e sulla sua *shelf-life* microbiologica. Questi punti sono stati affrontati in quattro contributi sperimentali la cui organizzazione e i principali risultati sono riassunti in seguito.

Nel **primo contributo** sono stati utilizzati 768 polli, di due genotipi (resa in petto standard o elevata), dei due sessi, e sottoposti a due sistemi di alimentazione (*ad libitum vs.* restrizione precoce, dal 13 al 21 giorno; restrizione quantitativa dell'80%) al fine di valutare le differenze di performance produttive, qualità della carcassa, e della carne. Inoltre, 192 *Pectoralis major* (4 per gruppo sperimentale) sono stati utilizzati per valutare il grado di degenerazione delle fibre muscolari (DFM) associata a quadri di *white striping* e *wooden breast* a differenti età (14, 21, 28, 35 e 46 giorni) e utilizzando la colorazione ematossina ed eosina per valutare la morfologia tissutale, la Masson tricromica per identificare il collagene, e la Oil red e Nile blu per i lipidi. Le carcasse appartenenti al genotipo a resa standard sono risultate più pesanti (2358 g *vs.* 2319 g; P<0.001) e con una minore resa di macellazione (73,6 *vs.* 74,0; P<0,001) rispetto al genotipo ad alta resa; inoltre la carne della linea standard presentava un pH più elevato (5,89 *vs.* 5,85; P<0,05) e minori perdite da scongelamento (10,5% *vs.* 9,43%; P<0,05). In quanto all'effetto del sesso, le femmine sono risultate più leggere (-24%) e con una minore resa di macellazione

(-7%; P<0,001). Gli animali sottoposti a restrizione alimentare avevano peso vivo finale inferiore (-2%; P<0,001) e minor resa di macellazione (-0,3%; P<0,05) rispetto a quelli non razionati. La percentuale di animali con DFM è risultata maggiore negli animali alimentati *ad libitum (*75,0% *vs.* 62,5%; P=0,01) ed è aumentata con l'età (18,8%, 28,1%;75,1%, 96,9% e 96,9% rispettivamente a 14, 21, 28, 35 e 46 giorni. A 21 giorni di vita, la percentuale di animali con DFM è risultata superiore nel gruppo che aveva ricevuto il mangime *ad libitum* rispetto a quelli che avevano appena terminato la restrizione alimentare (50,0% *vs.* 6,3%; P<0,01), tuttavia questa differenza è scomparsa con la re-alimentazione a 35 giorni (100% *vs.* 93,8%. A livello istologico, i tessuti affetti da DFM a 46 giorni si sono caratterizzati per una perdita della striatura delle fibre, abbondanti processi necrotici, presenza di fibre degenerate circondate da neutrofili e macrofagi sparse in mezzo ad abbondante collagene e adipociti; le fibre necrotiche hanno inoltre mostrato un'alta percentuale di nuclei apoptotici.

Nel **secondo contributo,** 900 polli di sesso maschile sono stati allevati fino alla macellazione (48 giorni) al fine di valutare l'effetto di due genotipi, Ross 308 e Cobb500, e tre sistemi di alimentazione (*ad libitum*-AL, restrizione precoce-RP, da 13 a 23 giorni, e restrizione tardiva-RT, da 27 a 37 giorni, con una restrizione all'80%) su prestazioni, qualità della carne, presenza di miopatie, e degenerazione delle fibre muscolari (DFM) a differenti età (22, 36, e 48 giorni). Durante tutta la prova il gruppo AL ha mostrato un maggiore velocità di crescita ed ha quindi raggiunto un peso finale più elevato rispetto agli animali sottoposti restrizione tardiva (3482 g *vs.* 3399 g; P<0.01), mentre il gruppo RP ha mostrato valori intermedi (3454 g). Inoltre, animali alimentati *ad libitum* hanno mostrato maggior resa in *P. major* (AL 26,4% *vs.* RT 25.4%; P<0.05). L'utilizzo di un sistema alimentare piuttosto che di un altro non ha influenzato la frequenza di *white striping* e *wooden breast*. Alla fine della prova, i polli Ross sono risultati più pesanti (3548 g vs 3342 g; P<0,001) raggiunto con un indice di conversione maggiore (1,69 *vs.* 1,62; P<0,001). Ancora, il genotipo Ross ha mostrato maggiore resa di macellazione (77,9% *vs.* 77,0%; P<0,001), ma minor incidenza di *P. major* (25,6% *vs.* 26,2%; P<0,05), oltre che maggiori pH (5,98 *vs.* 5,89; P<0,001), perdite di cottura (26,3% *vs.* 24,5%; P<0,01) e sforzo di taglio (2,39 kg/g *vs.* 2,12 kg/g; P<0,05). La frequenza di petti con *white striping* severo è risultata maggiore nei Ross rispetto ai Cobb (25,9% *vs.* 7,41%; P<0,001), mentre la presenza di *wooden breast* non è stata influenzata dal genotipo o dal sistema di alimentazione. Il grado di DFM è aumentato significativamente con l'età dei polli (1,25 a 2,42 dal 22 al 48 giorno; P<0,001), ma non alla fine della prova non è stato condizionato dal sistema di alimentazione. Diversamente, la DFM è risultata minore nei Cobb che nei Ross (1,67 *vs.* 2,03; P<0,001).

Nel **terzo contributo,** 800 polli da carne di due genotipi (Cobb 500 e Ross 308), di entrambi i sessi, sono stati sottoposti a due fotoperiodi: 14 ore di luce e 10 di buio *vs.* 18 ore di luce e 6 di buio. Alla fine della prova i polli Cobb sono risultati più pesanti rispetto ai Ross (+4%; P<0,001); questa differenza è dipesa da un diverso accrescimento (72,5 *vs.* 70,0 g/d; P<0,01) e da una diversa ingestione di alimento (+5%; P<0,001) nei due tipi genetici; tuttavia, l'indice di conversione è risultato essere peggiore nei Cobb

rispetto ai Ross (1,61 *vs.* 1,63; P<0,01). Coerentemente con i risultati di prestazioni produttive, le carcasse dei Cobb sono risultate più pesanti (2366 g *vs.* 2276 g; P<0,001) e hanno presentato una maggiore incidenza del *P. major*. Inoltre, la carne dei Cobb è stata caratterizzata da minori perdite di cottura (27,0% *vs.*29,3%; P<0,001) e minore sforzo al taglio (2,29 kg/g *vs.* 2,55 kg/g; P<0,01). Infine, la frequenza di miopatie è risultata simile nei due genotipi (76,0% e 77,9 per WS; 6,25% e 6,25% per WB). Le femmine a fine prova sono risultate più leggere rispetto ai maschi (2853 g *vs.* 3511 g; P<0,001) e con un peggiore indice di conversione (1,64 *vs.* 1,59; P<0,001). In maniera coerente con le differenze di peso vivo, le carcasse delle femmine sono risultate più leggere di quelle dei maschi (2063 g *vs.* 2580 g; P<0,001), con una minore resa di macellazione (73,7% *vs.* 74,5%; P<0,05), e una maggiore incidenza di *P. major* (25,6% *vs.* 24,2%; P<0,001). Le femmine hanno mostrato valori più bassi nel pH della carne (5,92 *vs.* 5,98; P<0,01), minori perdite di cottura (26,4% *vs.* 29,9%; P<0,001), minore sforzo al taglio (2,26 kg/g *vs.* 2,58 kg/g) e maggior contenuto di proteine (21,6% *vs.* 20,7%; P<0,001). Infine, la percentuale di WS e di WB è risultata inferiore nelle femmine rispetto ai maschi anche se in maniera non significativa (70,5% *vs.* 83,3% e 3,13% *vs.* 9.38% rispettivamente per WS e WB). Il diverso fotoperiodo ha influenzato il peso vivo degli animali: già a 16 giorni i due gruppi sperimentali avevano pesi significativamente differenti e tale differenza si è mantenuta fino alla fine della prova (3130 g *vs.* 3233 g; P<0,001), come conseguenza di un accrescimento e un consumo alimentare inferiori nel gruppo con fotoperiodo più breve che ha anche mostrato un migliore indice di conversione (1,61 *vs.* 1,62; P<0,01). Infine, il fotoperiodo breve ha avuto positivi effetti sulle miopatie, riducendo la frequenza di petti con WS (64,6% *vs.* 89,0% rispettivamente per 14L e 18L; P<0,001) e di petti con WS severo (18,8% *vs.* 37,8% per 14L e 18L). Gli animali allevati con il fotoperiodo breve hanno mostrato anche carni più tenere (2,20 kg/g *vs.* 2,64 kg/g; P<0,05) e con un minore contenuto di grassi (1,87% *vs.* 2,29%; P<0,05). La presenza di miopatie ha modificato anche alcune caratteristiche della qualità della carne: petti affetti da WB hanno presentato pH più elevato (5,92 *vs.* 5,98; P<0,05), maggiori perdite di cottura (34,2% *vs.* 27,6%; P<0,001) e maggiore sforzo di taglio (3,70 kg/g *vs.* 2,90 kg/g; P<0,01) rispetto a petti normali. Inoltre l'analisi chimica ha dimostrato che i petti con WB presentavano un maggiore contenuto di lipidi (1,82% *vs.* 2,53%; P<0,01) e un minore contenuto proteico (19,8% *vs.* 21,4%; P<0,001) rispetto a petti normali.

Nel **quarto contributo**, 48 petti normali**,** 48 petti affetti da *white striping* e 48 petti affetti da *wooden breast* sono stati conservati per 11 giorni a 4°C e sono stati esaminati a 24, 72, 120, 168, 216, 264 ore *post-mortem* al fine di valutare gli effetti delle miopatie sulla qualità della carne e sulla *shelf-life*. I petti normali hanno presentato minori pH e perdite di cottura rispetto a WS e WB (22,0% *vs.* 23,8% *vs.* 26,9%; P<0,001). Inoltre, i petti normali e quelli affetti da WS hanno mostrato un contenuto maggiore di proteine rispetto a WB (23,9% e 23,2% *vs.* 21,4%; P<0,001) e minore contenuto di estratto etereo rispetto ai WB (1,09% *vs.* 1,88%; P<0,001). Nelle carni normali, sono stati osservati un più alto contenuto di acidi grassi saturi (31,3% *vs.* 28,0% di media) e un minor contenuto di acidi grassi insaturi rispetto a WS e WB (68,7% *vs.* 72,0%; P<0,001). Le differenze sono state attribuite soprattutto alle variazioni degli acidi grassi polinsaturi. Durante la conservazione, le caratteristiche reologiche e chimiche delle carni normali e

di quelle affette da miopatie hanno mostrato un'evoluzione simile. Tuttavia, i petti normali sono stati caratterizzati da una maggiore conta microbica iniziale (TVC) che ha determinato un accorciamento nella *lag phase* rispetto a WS e WB (46,3 h *vs.* 85,2 h e 77,8 h). La soglia di *shelf-life* (7 $\log_{10}$ CFU TVC/g) è stata raggiunta più velocemente dai petti normali (130 h) rispetto ai WS (149 h) e WB (192h). Inoltre, TVC e *Pseudomonas* spp. sono risultati significativamente più alti nelle carni normali rispetto a quelle alterate tra le 72 ore e 216 ore di conservazione.

In conclusione, sulla base dei risultati della presente tesi, le strategie alimentari sono efficaci per il controllo e la riduzione del tasso di crescita e, quindi, per la riduzione delle degenerazioni muscolari e della frequenza di miopatie nei polli da carne fintanto che gli animali sono sottoposti a restrizione alimentare. Tuttavia, quando la restrizione viene interrotta e i polli possono manifestare accrescimento compensativo, l'effetto positivo viene perso. D'altra parte, se la restrizione è realizzata fino alla fine del periodo di allevamento, riducendo la durata del fotoperiodo, o se è praticata in un periodo tardivo, la riduzione della frequenza/severità delle alterazioni è accompagnata da una perdita di prestazioni produttive e risultati di macellazione. Inoltre, si conferma l'effetto importante del sesso, con i maschi caratterizzati da maggiore velocità di accrescimento, ma anche da una maggiore suscettibilità alla comparsa di *wooden breast* rispetto alle femmine. In quanto ai genotipi testati, tutti sono sensibili e soggetti alle miopatie. Tuttavia, quanto minore è il tasso di crescita, tanto minore risulta la frequenza delle alterazioni e/o della severità delle alterazioni a livello del petto. Infine, le carni affette da miopatie presentano qualità reologica, tecnologica e nutrizionale inferiori rispetto alla carne normale, ma la shelf-life microbiologica delle carni refrigerate risulta più favorevole in presenza di miopatie.

ABSTRACT

Poultry production is one of the few animal sectors still growing due to the high consumer request, guaranteeing high production level and a standardized product. However, the selection of fast growing strains with high breast yield has brought about some defects affecting the meat quality. The alterations most observed under commercial condition, which do not represent an issue for human health but are an economic loss for the stakeholders, are white striping and wooden breast. These abnormalities, which alter the chemical and technological meat quality requiring a further transformation or the destruction of the product, affect especially the most valuable cut, the breast. Despite recent investigations, knowledge about the precise etiology and, especially, the factors that could modify the occurrence of these alterations is still lacking.

With this background, the aims of this PhD project were: i) to evaluate the feeding strategies that could affect performance, occurrence and severity of myopathies and meat quality of broiler chickens; ii) to elucidate the gender effect on the incidence of white striping and wooden breast and on meat quality of broiler chickens; iii) to compare different genotypes in relation to myopathy occurrence and meat quality; iv) to assess meat quality traits and microbial shelf-life in abnormal meat. These points were developed in four experimental contributions which working plan and main results are summarized below.

In the **first contribution,** 768 broiler chickens, two genotypes (standard breast yield and high breast yield), both genders were submitted to two feeding strategies(*ad libitum vs.* early feed restriction, 13 d to 21 d; restriction rate at 80%) to evaluate productive performance and, especially, carcass traits and meat quality. Moreover, to investigate muscle fibre degeneration (MFD) associated with white striping and wooden breast, 192 *Pectoralis major* (4 animals for experimental group) were sampled at different ages (14, 21, 28, 35, and 46 d) for histological analyses by hematoxylin and eosin (H&E) staining to evaluate tissue morphology, Masson's trichrome to identify collagen presence, and Oil red and Nile blue for lipid presence. Standard-yield chickens resulted to have significant higher carcass weights (2,358 g *vs.* 2,319 g; P<0.001) and lower dressing out percentage (73.6% *vs.* 74.0%; P<0.01) compared to high-yield birds, and higher final meat pH (5.89 *vs.* 5.85; P<0.05) and thawing losses (10.5% *vs.* 9.43%; P < 0.05). The effect of gender was clear especially on carcass traits: females showed lower carcass weight (-24%), dressing percentage (-0.7%; P<0.001) than males. Finally, the effect of feeding system appeared on carcass traits: restricted birds had lower carcass weight (–2%; P<0.001) and dressing percentage (-0.3%) (P<0.05) than those always fed *ad libitum*. Moreover, gender and feeding restriction affected meat final pH, lower in *ad libitum* group than in restricted one and in females than males. The MFD was higher in *ad libitum* than restricted chicks (75.0% *vs.* 62.5%; P=0.01) and increased with age (18.8%, 28.1%, 75.1%, 96.9%, and 96.9% at 14, 21, 28, 35, and 46 d). At 21 d broilers fed with *ad libitum* diet had higher MFD occurrence than in those under restriction (50.0% *vs.* 6.3%; P<0.01); this variation disappeared at 35 d (100% and 93.8%). The number of microvessels decreased with age (20.7 to 9.46; P<0.001) and the number of nuclei

positive to the anti-cleaved lamin A antibody increased. At histological analyses, MFD at 46 d was characterized by loss of typical cross striations, massive necrotic process, degenerating fibres surrounded by neutrophils and macrophage, scattered fibres in an abundant collagen tissue, and adipose cells; necrotic fibres showed a high percentage of apoptotic nuclei, and regenerating fibres appeared positive to anti-PCNA antibody. In conclusion, slaughter results and carcass traits changed especially with genotype and gender, coherently with slaughter weight whereas meat quality was mostly affected by genotype; MFD soon occurred after 2 weeks of growth and increased dramatically within 28 d. Early feed restriction reduced MFD as long as animals were restricted, but no residual effect was recorded after re-alimentation.

In the **second contribution,** 900 male chickens were reared until slaughter (48 d) to evaluate the effect of two genotypes (Cobb *vs.* Ross) and three feeding regime (*ad libitum*-AL *vs.* early restricted-ER, 13 to 23 d of age, and late restricted-LR, 27 to 37 d of age; restriction rate at 80%) on productive performance, meat quality, occurrence of myopathies, and muscle fiber degeneration (MFD) at different ages (22 d, 36 d, and 48 d). During the whole trial AL group had higher growth rate and reached higher final live weights (3,482 g *vs.* 3,399 g; P<0.01) compared LR birds resulting in higher incidence of *P. major* (AL 26.4% *vs.* LR 25.4%; P<0.05), whereas ER chickens had intermediate value (3,454 g and 25.6%). At gross examination, the feeding restriction program did not affect the incidence of WS and WB.
At 48 d, Cobb chickens presented lower weight than Ross (3,342g *vs.* 3,548 g; $P < 0.001$) with lower feed conversion rate (1.69 *vs.* 1.62; P<0.001). The Cobb birds also had lower dressing out percentage (77.0% *vs.* 77.9%; *P*<0.001), but higher *P. major* proportion (26.2% *vs.* 25.6%; *P*<0.05), besides lower pH (5.89 *vs.* 5.98; P<0.001), meat cooking losses (24.5% *vs.* 26.3%; *P*<0.01), and shear force at *P. major*. (2.12 kg/g *vs.* 2.39 kg/g; P<0.05) The occurrence of severe WS breasts was higher in Ross than in Cobb (25.9% *vs.* 7.41%; P<0.001). The WB occurrence was not affected by the feeding system or the genotype. The MFD score significantly increased with the chicken age (1.25 to 1.88 and 2.42 from 22 d to 37 d and 48 d of age; P<0.001). Finally, MFD score was not affected by the feeding system, but differed between genotypes (1.67 *vs.* 2.03 in Cobb *vs.* Ross; P<0.001).

In the **third contribution,** 800 chicks, half Cobb 500 and half Ross 308, both genders were kept under a daily lighting program of 18 hours of light and 6 of dark (18L:6D) or under a 14 hours of light and 10 hours of dark (14L:10D). Females at the end of the trial were significantly lighter than males (2,853 *vs.* 3,511 g (P<0.001). Feed intake averaged 105 g/d in females and 125 g/d in males (P<0.001) on the whole trial, which corresponded to a worse feed conversion in females than males (1.64 vs 1.59; P<0.001). Coherently, carcasses were lighter in females than males (2,063 g *vs.* 2,580 g; P<0.001) as well as dressing out percentages (73.7% *vs.* 74.5%; P<0.05). Females showed higher proportion of breast (40.9% *vs.* 39.0%; P<0.001) and *P. major* (25.6% *vs.* 24.2%; P<0.001). Gender also affected many quality traits measured on *P. major*: the females showed lower ultimate pH values (5.92 vs 5.98; P<0.01), lower cooking losses (26.4% vs 29.9%; P<0.001), lower shear force (2.26 kg/g vs 2.58 kg/g; P<0.01), and higher crude protein content (21.6% vs 20.7%; P<0.001) than males. The occurrence of WS (70.5% vs

83.3%) and WB (3.13% vs 9.38%) was lower (P<0.05) in females compared to males. Cobb 500 chicks resulted heavier than Ross 308 (P<0.001). During the whole trial, feed intake was higher (P<0.001) and the conversion index worse (1.61 *vs.* 1.63; P<0.01) in Cobb than in Ross. The carcass weights were higher in Cobb (2,366 g *vs.* 2,276 g; P<0.001) which had higher *Pectoralis major* muscles (25.8% *vs.* 24.0%; P<0.001) than Ross chickens. Moreover, at *P. major*, Cobb showed lower cooking losses (27.0% vs 29.3%; P<0.001) and shear force (2.29 kg/g vs 2.55 kg/g; P<0.01) compared to Ross. Finally, genotype did not influence the occurrence of myopathy, which was similar for WS (76.0% and 77.9%) and WB (6.25% and 6.25%) in the two genotypes. The different light programs affected chicken live weight: at 16 d of age live weight was lower under 14L than 18L program (617 g vs 644 g; P<0.001). The difference of live weight was maintained until 45 d (3,130 g vs 3,233 g; P<0.001). The gap in feed consumption was relevant from the second week resulting in a better conversion index for 14L group in the whole trial (1.61 *vs.* 1.62; P<0.01). The light program 14L also produced a significant reduction in the incidence of WS (65% to 89% of the controlled animals; P<0.001) and of severe WS (from 37.8% to 18.8%; P<0.01), but WB did not change. The light program has also affected the *P. major* quality; in particular, the breasts of animals reared with 14 hours of light requested a significantly lower shear force than the chickens reared with the module 18L (2.20 kg/g *vs.* 2.64 kg/g; P<0.05). The fat content was lower in breasts of chickens reared with 14 hours of light (1.87% vs 2.29%; P<0.05). The presence of myopathies modified quality traits: WB breasts showed higher pH (5.92 vs 5.98; P<0.05), increased lipid content (1.82% vs 2.53; P<0.01), and lower protein content (19,8% *vs.* 21,4%; P<0.05) than normal breasts. Finally, WB also increased cooking losses (34.2% to 27.6%; P<0.001) and shear force (3.70 kg/g to 2.90 kg/g; P<0.01).

In the **fourth contribution,** 48 normal, 48 white striped (WS), and 48 wooden (WB) breasts were stored in a refrigerated cabinet for 264 h d at 4°C using PVC film; then they were analysed at 24, 72, 120, 168, 216, and 264 h post-mortem to evaluate the impact of emerging myopathies on meat quality and microbial shelf life. Normal breasts exhibited lower weight ($P < 0.001$) and cooking losses (22.0% *vs.* 23.8% *vs.* 26.9%) than WS and WB. Normal breast showed higher protein content than WB (23.9% and 23.2% *vs.* 21.4%; $P < 0.001$) and lower ether extract content than WB (1.09% *vs.* 1.88%; $P < 0.001$) with intermediate values for WS. Normal breasts exhibited higher saturated fatty acids (FA) (31.3% *vs.* 28.0% on average) and lower unsaturated FA rates (68.7% *vs.* 72.0%) than WS and WB ($P < 0.001$). Differences were mainly due to polyunsaturated FA (30.5% in normal *vs.* 35.3% and 35.4% in WS and WB; $P < 0.001$). During storage, technological and chemical traits of normal and affected meat showed a similar pattern. Nevertheless, normal breasts had the highest initial total viable count (TVC) and the shortest TVC lag phase than WS and WB (46.3 h *vs.* 85.2 h and 77.8 h). Microbial shelf life threshold (7 $\log_{10}$ CFU TVC/g) was achieved first in normal (130 h) than in WS (149 h) and WB (192 h).

In conclusion, based on results of the present thesis, feeding strategies can be an efficient method to control and reduce the growth rate of chickens and, thus, to reduce the occurrence of muscle fiber

degeneration and myopathies, as long as the animals are kept under restriction, but when this regime is interrupted, and the chicks show compensatory growth, the positive effort is lost. Nevertheless, if restriction is performed until the end of the trial, e.g. by reducing photoperiod, or if performed late in the growth, the reduction in occurrence/gravity of these alterations is at the expenses of productive performance. Gender has been confirmed to play a fundamental role in poultry production. Males have higher performance but are more prone to show wooden breasts compared to females, whereas differences in white striping are not relevant. All commercial genotypes tested are subjected to myopathies; nevertheless, the lower the growth rate, the lower the occurrence of myopathies. Finally, abnormal meat have lower rheological, technological, and nutritional properties compared to normal meat. However, microbial shelf life under aerobic conditions is longer in abnormal meat compared to normal meat.

CHAPTER 1: LITERATURE REVIEW AND AIMS

Introduction

Main features of poultry production

In the last 50 years the demand and the consumption of chicken meat has been characterized by a huge increase and the forecast for 2025 shows a tendency towards further increases. Indeed, poultry meat production has gained the second place in global meat production, after swine. The reasons for its success are quite different: first, the improving of farming facilities in developing countries, which in the last 30 years have overcame industrialized countries; second, the low cost of production; third, the fast productive cycle, and last, but not least, the versatility of this meat.

World poultry meat production increased from 1.97 million of tons in 1960 to 90.4 million in 2016 (Figure 1.1) (USDA, 2017). The major producers of broiler meat are USA, Brazil, China, and the EU. The United States of America produce the 20.7% of the world production followed by Brazil with 15.3%.

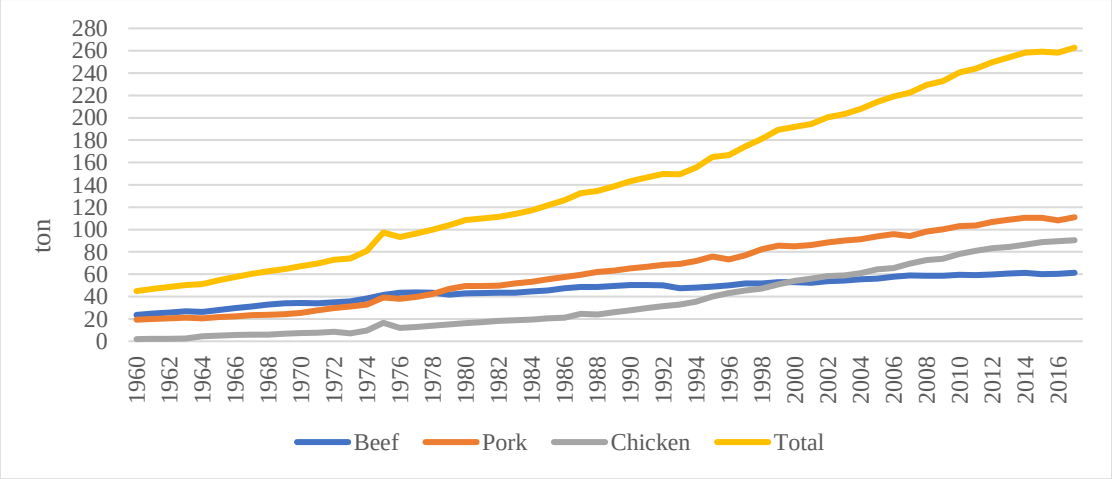

Figure 1.1 World meat production in the years (USDA, 2017).

The European Union is one of the most important poultry meat producer contributing with the 12% of production and especially Poland, France, Germany, England, Spain, and Italy play a central role. Furthermore, Europe is self-sufficient covering 104% of its own requirements. The total EU production of poultry meat is 14.4 million of ton per year and 11.5 million are from broiler chickens. According to the global trend, European production and consumption are still rising. In 2015 the consumption of poultry meat was 22.5 kg per-capita of which 18.4 kg from broiler chicken meat (FAO, 2016).

Usually chickens are reared in intensive systems to increase the production and standardize the quality. Under this type of rearing, different products are obtained according to the length of the productive cycle, the gender, and the bird final weight. However, the duration of the cycle is always quite short, 10 weeks at maximum for the heavy chickens. Thus, it is possible to have more cycle in the same poultry-house during a year.

Farms usually have one or more rectangular plants with 14-16 m of width and variable length, depending on the number of reared animals. The walls could be "open or close" depending on the presence of windows which could serve specific lightening programs and/or the air flow. All the principal environmental aspects, like temperature, humidity, and light are always controlled and recorded. The full concrete ground is generally covered with litter of 5-10 cm of thickness, depending on materials used and cycle length (i.e. shaving of white wood, straw, and rice hull and husk) (Guarino, 2015).

The litter quality represents a key point in the rearing of these animals because a wrong management could cause the occurrence of pathologies (e.g. dry respiratory problems if the litter is too dry) or altered behaviors (sitting due to footpad lesions). The air quality is another parameter always controlled. Indeed, inside each poultry-house a regular air flow should be guaranteed according to the number of animals and their live weight to avoid thermal stress, intoxication by dangerous gases which could be responsible of the disease occurrence. Besides intensive ones, other rearing systems are outspread:

- extensive indoor rearing: where the animals are reared in a close building with a density of 12 animals/m^2 (maximum of 25 kg/m^2). In these facilities, the animals are usually slaughter at 65 days.
- Free range: 13 animals/m^2 (maximum of 27.5 kg/m^2). Animals should spend at least half of their life outside. The minimal age to slaughter is 56 days.
- Open-air: 12 animals/m^2 (25 kg/m^2) in fixed shelter and 20 animals/m^2 (maximum of 40 kg/m^2) in mobile shelter; the outside access must be guaranteed from the sixth week of life guaranteeing at least 1 m^2 of open-air area per animal. The minimum slaughter age is 81 days (Reg. (CEE)N.1538/91 D.M.465 10/09/1999).
- Organic: 10 animals/m^2 and at least one third of bird life is outside with an available surface of 4 m^2 per bird. The minimum slaughter age is 81 days. An organic diet must be used containing at least 60% of raw materials produced by the same farm (CE N.834/2007 and N.889/2008 D.M.18354 27/11/2009).

In the modern poultry industry, the farmed animals are not anymore pure breed, but they are the result of commercial crossbreeding, usually named as "hybrids", which had been selected for specific features. The genetic improvement in the broiler sector has achieved higher level respect to other species due to the extremely shortness of the reproductive and productive cycles (Bittante *et al.*, 2005).

In the 1950-1960, the breeds were principally with twofold aptitude to produce meat and eggs: the breeds most reared were: *American Plymouth Rock*, characterized by a large breast and high weights (the males

weight 3 kg); *American New Hampshire*, selected for the high growth rate and a large breast, *Delawere*, quite similar to the *Plymouth Rock; Cornish*, a heavy chicken, the male weighted around 3.5 and 4.5 kg with a large breast and heavy bones.

The principal objectives for the selection in broiler production have been the improving of feed conversion, growth rate, and breast yield. However, some negative features are associated to these improved traits, like as the higher susceptibility to the diseases and leg abnormality (Bittante *et al.*, 2005). Indeed, animals selected for heavy weight show a weak skeleton which affect the ability of the animals to move, and thus, they are forced to resting on the floor. The limits of birds to move in response to leg abnormalities and the high stocking densities used under intensive system affect the welfare of the animals which are more subjected to various disease, like dermatitis, ascites, and metabolic disease (De Jong *et al.*, 2012).

Currently, broiler chickens for meat production are produced mainly by two multinational industries: the Aviagen group, which sells the ROSS genotypes, and the COBB-Vantress group, which produces the COBB genotypes (Aviagen, 2015; Cobb-Ventress, 2015).

Main quality features of chicken meat

Chicken meat is worldly recognized as a healthy food with high-quality nutritional value. Indeed, it appears to have reduced fat and cholesterol contents respect to the others meat: it contains 70% of water, 20% of proteins, and 4% of lipids on average. However, the percentage of lipids is not so different from other species, but the fat localization is principally abdominal and under the skin and, thus, it can be easily removed reducing the percentage of the lipids in the meat (Zaniboni and Cerolini, 2015). Furthermore, this meat is characterized by a good level of proteins with high nutritional value due to the high presence of essential amino acids, like arginine and lysine, and to the low collagen content (Mudalal *et al.*, 2014). Poultry meat is usually defined as “white meat” because of the low presence of deoxymyoglobin, oxymyoglobin and metamyoglobin.

For poultry meat, as for the other meats, the traits which can affect its quality are principally pH, water holding capacity, color and texture. The pH plays a fundamental role in the transformation from muscle to meat. During the life, the muscle pH is around 7.0 and 7.2, but after the animal death it decreases rapidly. Indeed, after death, the muscle continues its contractile activity, but the lack of blood supply to the muscle and so, the lack of oxygen, leads to the activation of glycolysis to produce energy. Thus, the glycogen that is stored inside the myocyte, the cell responsible of the contractile activity, is converted into lactic acid and energy. The accumulation of the acid leads to a decrease in the inner muscle pH. When the cell consumes all the stored glycogen, the contractile activity ends and the muscle enters in *rigor mortis*. In poultry, this situation usually begins 45 minutes after death and starts from the head to the legs (Zaniboni and Cerolini, 2015).

Then, the resolution of *rigor mortis* depends on different factors which can be extrinsic or intrinsic. The *rigor mortis* in avian species tends to be resolved faster than in mammalians because the body temperature is higher, and the *post-mortem* muscular activity is stronger than in other species. This decrease of the pH is due by an accumulation of lactic acid into the cells, which leads to the activation of specific endogenous proteases, calpain and cathepsins. These proteins are responsible for the degradation of the Z line of the skeletal muscle cells and consequently for the collapse of the actin-myosin complex (Brad Kim *et al.*, 2014). When this phase ends usually the pH at breast level is around 5.7-5.9.

Water holding capacity (WHC) is another important factor for the quality of the meat. This aspect defines the capacity of the meat to entrap the water inside the cells and is strongly affected by the ultimate pH and by the amount of space between the cells in the muscle. Also, other factors can influence the water holding capacity of meat, like genetics, live animal handling and, early post-mortem temperature management. All these factors have the potential to affect the rate and the decline of the pH. Meat WHC is an important factor for the forward transformations, because meat with too low WHC loses more water during cooking resulting in low-quality processed products.

The last parameter important for the quality is the color. Usually the color is measured by the *CIELab method* which is based on three different coordinated: 1) L index: the lightness index. The range of this coordinate is from 0 to 100 where 0 is black and 100 is white but usually in poultry meat ranges from 40 to 60; 2) a* index. It represents the red/green coordinate. Negative values are for green and positive values are for red. In the poultry meat the range of this coordinate is from 0 to 4.; 3) b* index. It represents the blue/yellow coordinate. Negative values are for blue and positive values are for yellow. In the poultry meat the range of this coordinate is from 15 to 22.

The texture of the meat is related to the shear force applied to the meat. The tenderness of the meat is affected by different intrinsic factors of the meat, like its chemical composition (in terms of moisture, protein, fat and collagen), the length and the diameter of the muscle fibres, the type of muscular fibres (type I, type IIa, type IIx and, type IIb), the amount of connective tissue, and the water holding capacity (Bruce and Ball, 1990). The tenderness of the meat could be affected also by extrinsic factors, like the early deboning and cooking methods (Cavani *et al.*, 2009).

In the last years, the concept of meat quality is more and more including the measure of meat shelf-life. This term refers to "*either the period corresponding to the period preceding the 'use by' or the minimum durability date"* (Commission Regulation No 2073/2005 15 November 2005 on microbiological criteria for foodstuffs, 2005). So, also the "durability" of the meat has become an aspect really important for the consumer.

Numerous extrinsic and intrinsic factors affect the shelf-life of the meat. The principal intrinsic factors are pH, water holding capacity, moisture content, the oxidation-reduction potential, nutritional content, antimicrobial constituent (Jay, 1998). The extrinsic factors, like temperature of storage, relative humidity,

presence and concentrations of gases, presence of other organisms which could produce substances to inhibit the activity of food organisms, act on the meat from the outside and could modify the characteristics and the conservation.

The poultry meat is a product highly perishable and usually its shelf-life varies between 4 and 10 d under refrigeration. To value the processing hygiene, storage quality and potential shelf life in aerobic condition are used some general indicators, like mesophilic aerobic counts, psychrotrophs, *Enterobacteriaceae*, coliforms, yeasts and molds. Another population responsible of meat degradation are the *Pseudomonadacee* which are the principal ones involved in change of product scent (Dawson *et al.*, 2013).

Principals alterations of poultry meat

In these years with the developing of genotypes characterized by fast growth an increase in the alteration of poultry meat was observed. Indeed, different studies affirm that modern fast-growth broiler genetic lines suffer of idiopathic myopathies and an increased susceptibility to stress-induced pathologies. In these animals an alteration in intracellular calcium homeostasis and consequent changes in sarcolemma integrity were observed and may be the result from hypertrophy of muscular fibres and the lack of correct vascular supply. These abnormalities most frequently affect the most valuable cut, the breast, and for this reason have been deeply studied. The myopathies most frequently observed are PSE-like), deep pectoral disease, white striping, wooden breast and spaghetti meat. The PSE-like (pale-soft-exudative) is a myopathy observed also in livestock animal and results in alteration of meat texture and color and presence of exudative liquid on the surface. The deep pectoral disease (DPD) is a myopathy described in the past which sometimes is reported currently; it is characterized by an altered green coloration of the *Pectoralis minor*. White striping (WS) is a quite recent described alteration: it affects principally the chicken breast and appears as white striations above the surface of the meat. Associated to WS occasionally is possible to found wooden breast (WB): The breast affected by WB results with hardened texture of some areas and bulging. The last myopathy observed under commercial conditions is spaghetti meat: only few data are present in literature, but it is defined as a loss of the meat structure.

PSE-like breast meat (Pale Soft Exudative)

The PSE alteration was identified for the first time in pig meat and was associated to a genetic single mutation in the ryanodine receptor of the sarcoplasmatic reticulum involved in calcium release; this mutation seems to be isolated in animals that are stress-susceptible and prone to developing PSE meat (Petracci and Cavani, 2012).Lately, this condition was detected, in a very similar way, also in turkey and chicken meat although no evidences of the genetic mutation have been described (Barbut, 1996, 1997; Van Laack *et al.*, 2000, Petracci and Cavani, 2012). The etiology of this alteration is not well known, it is assumed that it is associated to a high stress level before the death of the animal. Nevertheless, two different categories of studies have been conducted to identify the cause: those that are studying the role carried out by the genetic selection and those occupied to define the effect of environmental factors. Indeed, some authors, like Berri *et al.* (2005), stated the selection of fast-growth breast yield lines could exert a positive effort on *post-mortem* acidification decreasing the glycogen storage and, in this way, reducing the production of lactic acid. Moreover fast-growth breast yield lines resulted to be more susceptible to heat stress compared to their predecessors due to a reduced thermoregulatory capacity. The thermal distress during the animal life causes the increase in free radical production which can compromise the sarcolemma integrity (Petracci and Cavani, 2012). The intracellular damage condition leads to a rapid decrease of muscle pH and if this value is too low

and the muscle temperature is high in early *post-mortem* (within 30 minutes after the animal death), the proteins begin a denaturation process, which leads to decreased WHC and meat discoloration. The breast affected by this alteration shows a great exudative water loss and a decreased technological yield (Duclos *et al.*, 2007). Although the rather soft texture of the raw meat, when PSE meat is cooked it tends to be less tender because of excessive exudation (Duclos *et al.*, 2007).

Deep Pectoral Disease (DPD)

This myopathy, known also as Oregon disease and green muscle disease, was described for the first time in the year 1968 in turkeys. With the genetic selection of animal characterized by fast growth rate, also the broiler chickens suffered of this degenerative myopathy. Usually, it is described like an ischemic necrosis of the deep pectoral muscle (*Pectoralis minor* or *supracoracoideus*) due to the incapacity of muscle mass to swell in response to physical changes. Indeed, the muscle, entrapped between an inelastic fascia and the sternum, during intense activity, like wing flapping, can increase its weight by about 20% due to the blood supply. When this situation happens, the blood vessels in muscle crush and the muscle becomes ischemic. The lack of oxygen forces the muscle to use the low quantities of glycogen stored inside the cell (fiber type IIb). When also glycogen ends, the cell begins the lipid peroxidation. During this phase, free radicals, which can affect the cell survival hitting the cell membrane, are produced which cause the tissue necrosis (Cavani and Petracci, 2012). At macroscopic inspection, swollen red-brown lesions appear which can become green in the successive phases. This myopathy may be mono or bilateral. Normally it does not affect the animal health and it is observed during the cut-up and deboning. Histologically, the lesions show a central mass of necrotic muscle fibres surrounded by a fibrous capsule with an inner reactive and granulating border. Outside the fibrous capsule a zone of normal and regenerating muscle or a zone where there is substitution by fibro-adipose tissue can be found. The necrotic fibres frequently had polymorphism and increased diameter, they are lightly eosinophilic and can be separated by an edematous liquid. The fibre nuclei are difficult to see or absent. Usually, inflammatory cells are absent except in the zone close to the capsule (Wight and Siller, 1980). This alteration causes a carcass downgrade and require that meat is submitted to further transformations. The incidence of this myopathy is quite low, just below 1%, and does not represent a concern for the public health. However, consumers do not accept it (Kijowaski and Konstanczak, 2009).

White striping (WS)

This myopathy was described by Kuttappan *et al.* in 2009 and is defined like a degenerative myopathy of breast fillets characterized by degenerative or atrophic muscular fibres, variability in fiber size, vascularization and lysis of the fibres, mild mineralization, occasional regeneration, infiltration of inflammatory cells in the interstitial space, lipidosis, and fibrosis (Petracci *et al.*, 2016) affecting in this way

the nutritional and technological properties of the meat and affecting negatively consumer's opinion (Kuttappan *et al.*, 2012b).

This alteration seems to be linked to an altered blood supply the breast muscle, due to the impaired growth of blood vessels due to the size of the muscle. In selected genotypes, myofiber diameter increases through hypertrophy; this change causes a marginalization and compression of the vessels increasing microischemia of the muscle (Petracci and Cavani, 2012). The correct regeneration of the areas is so inhibited by the lack of blood which is important for the satellite cell activity. Furthermore, vascular endothelial cells promote the proliferation of muscle through the secretion of different growth factors. These cells are extremely responsive to the action of stimulatory or inhibitory growth factor like fibroblast growth factor. So, after the degeneration, the regeneration of the fibres should be done by the activation, the proliferation, and differentiation of satellite cells but without the correct growth factors the regeneration phase is altered, and the tissue cannot return to the normality (Velleman, 2015). Macroscopically, white striping appears as white stripes parallel to the direction of the muscle fibres over the *Pectoralis major*, due to a storage of lipids and connective tissue, like collagen, in the *interstitium* (Petracci *et al.*, 2015). Three different degrees of this myopathy have been described (Figure 1.2):

- Moderate: small white lines with less than 1 mm of thickness principally in the cranial part of the muscle. Usually, fillets affected by moderate WS are commercialized because WS presence does not downgrade the product for the consumer.
- Severe: large white lines with a thickness of 1-2 mm all over the surface. The meat affected by severe WS must be transformed into ready-to-cook or into ready-to-fork products
- Extreme: white bands with thickness superior of 2 mm covering all the surface of the muscle. At the slaughterhouse, affected breasts are thrown away because often associated with other myopathies, like *wooden breast* or *spaghetti meat*.

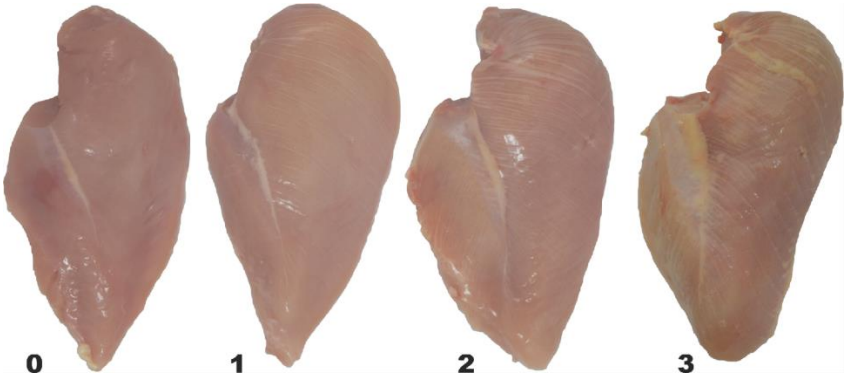

Figure 1.2. Classification of degrees of white striping on *Pectoralis major* **0=** Normal; **1**=Moderate; **2**=Severe; **3=**Extreme (Kuttappan *et al.*, 2016).

Indeed, Soglia *et al.* (2016) reported that also breast visually normal, histologically showed evidences of myodegeneration. However, if this alteration is not observed at macroscopic examination, the analyses of the serum enzymes, like creatine kinase, which is one non-specific indicator of muscle damage, can support this

thesis; indeed, Kuttappan *et al.* (2013) reported that animals with severe degree of *white striping* also presented high level of enzymes related to muscle damage. In fact, in animal affected by this myopathy, an increased level of creatine kinase, a protein used as indicator of muscle damage, has been observed, whereas creatine kinase is usually found within the muscle fiber, in which it is used to regulate the majority of cellular pathways, in particular those involved in signal transduction (Velleman, 2015).

Regarding WS occurrence, Kuttatappan *et al.* (2012b) reported that more than 50% of the fillets inspected at slaughterhouse may be affected. Unlike, Petracci *et al.* (2013) reported 12% of WS breasts in an Italian slaughterhouse. More recently, Tijare *et al.* (2016) found the average WS occurrence on flocks higher than 90%.

The quality of the meat is another aspect that could be affected by the presence of WS. Indeed, some authors reported that this myopathy could change the fillet water holding capacity and the meat pH (+0.7%) which could affect dripping losses, cooking losses, and shear force in further processed products (Kuttappan *et al.*, 2013). Analysing the chemical composition of meat affected by WS, breast fillets showed an increase in fat content, with a corresponding decrease in the percentage of protein. The degeneration of the muscular fibres observed at histological level could explain the reduction of the protein content. The increased fat content depends on lipidosis observed as a consequence of muscle fibres necrosis in affected meat (Kuttappan *et al.*, 2012).

Wooden breast (WB)

Wooden breast (WB) is a myopathy observed and described for the first time by Sihvo *et al.* in 2014 in Finland. Nowadays, the precise etiology of this alteration is still unknown as well as the patho-mechanism but different hypotheses have been suggested.

Macroscopically, WB affected *Pectoralis major* muscles show a remarkably hardened consistency diffused all over the surface or diffused on focally extensive areas. The hardened parts are pale and with outbulging areas (Figure1.3).

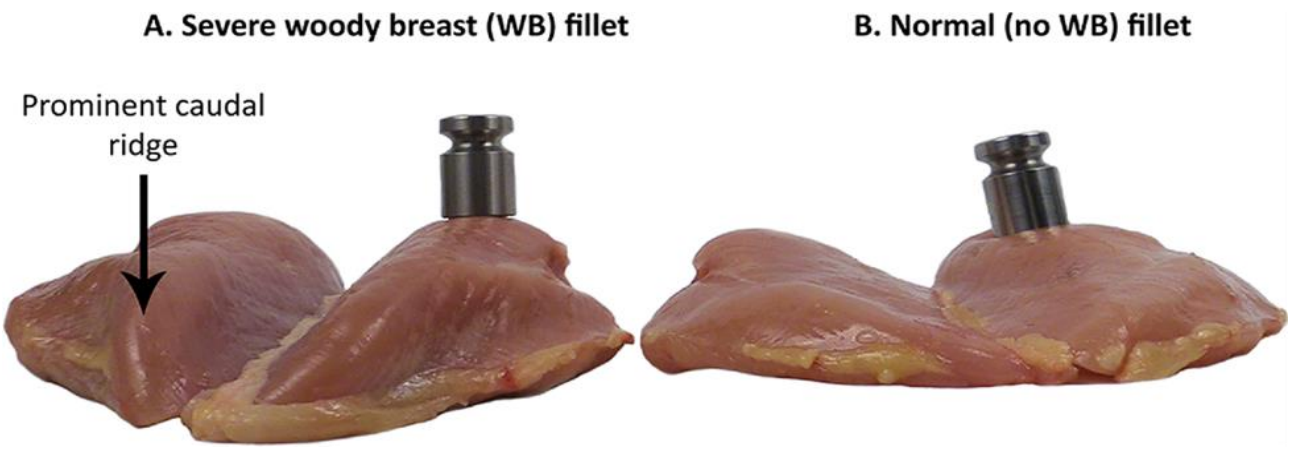

Figure 1.3. Comparison of severe wooden breast (WB) and normal fillets (Kuttappan *et al.*, 2016).

Sometimes on the surface a thick layer of liquid, moderately viscous, is present, as well as petechiae and small hemorrhages. Parallel white striations of 3-5 mm of width (severe degree of WS) may be associated to wooden breast myopathy. The histologic cross section of affected tissues is characterized by myofibres of variable diameter and fibres which have lost their typical polygonal structure. Split fibres, multifocal degeneration, necrosis, presence of fragmented hypereosinophilic amorphous fibres, loss of the typical striation of the fibres, and infiltration of inflammatory cells, principally represented by macrophages and hetherophils, are also observed. The tissue is often accompanied by thin fibres, slightly basophilic, central oval nuclei in a row, which represent the regeneration of the tissue. A moderate amount of granulated tissue or fibrosis is observed in the *interstitium*. Moderate to severe edema and accumulation of liquid which contains collagen material and inflammatory cells are reported (Sihvo *et al.*, 2014). The degree of fibrosis is more severe than in white striping. The WB incidence may largely vary from 41% (Sihvo *et al.*, 2017) to 53% (Dalle Zotte *et al.*, 2017).

Fillets affected by WB usually are heavier than normal fillets and exhibit greater cross-sectional area. Moreover, the meat affected by this alteration is characterized by high pH; also, the color values are modified by the presence of this myopathy, and all these changes are maintained also after the frozen storage. Dalle Zotte *et al.* (2017) reported that greater values of pH, a* and b* indexes were found in the cranial portion of affected fillets, the part which is most affected by WB. Other authors suggested that the reason of this phenomena could due to the thickness of the tissue. Indeed, the cranial area of the *Pectoralis major* is subjected to overstretching and ischemia, which result in a tissue damage and reparative responses (Kuttappan *et al.*, 2013). The higher ultimate pH observed in the WB meat may be explained by the strong negative correlation between the storage of glycogen and the weight of breast fillets, described by Le Bihan-Duval *et al.* (2008). Indeed, greater size of breast muscle is associated with a reduced glycolytic potential, resulting, in the end, in higher value of the pH in the *post mortem*. Soglia *et al.* (2016) speculated that these values of the meat ultimate pH could be linked to an alteration in the glycogen utilization, which could lead to a glycogen depletion.

According to Dalle Zotte *et al.* (2017), the higher values of a* index is probably related to a higher content in myoglobin storage: the tissue affected by WB is subjected to continuous regenerations phenomena, which could lead to a higher expression of a particular gene responsible of the myoglobin production. Regarding the b* index, meats affected by WB seem to be more yellow than normal meat, as reported by Mudalal *et al.* (2015) and Dalle Zotte *et al.* (2017). Also the water holding capacity is influenced by the presence of this myopathy; in fact, fillets with WB have lower WHC, and, consequently, superior cooking losses. As for WS, the explanation of this condition can be found in the histological aspect: the degeneration of the protein lead to a substitution of the fibres with collagen tissue and therefore the impossibility of the tissue to contain the water. Moreover, WB has a negative effect on proteins and lipids because induce the oxidation of these cellular components which results in reduce storage stability of the meat and, thus, WHC. The WB also

influences the meat texture: Soglia *et al* (2016) reported that fillets with WB were gummier and harder to be chewed than normal ones. Nevertheless, this aspect is not always consistent among trials because some authors refer that the palpatory hardness does not correspond to a major shear force, assuming that the extensive poor cohesion and the tendency of the fiber to separate could mitigate the hardness (Tasoniero *et al.*, 2016)

Spaghetti meat (SM)

In the last few years another muscle abnormality has been detected at the slaughterhouse which has been called *Spaghetti* meat (Baldi *et al.*, 2018). It appears as an alteration of the structural integrity of the cranial surface of the *Pectoralis major* muscle. A similar myopathy had been described in the turkey by Swatland (1990). In these animals an increase of cross-sectional area was described and the author assumed that that the fibres of the muscle had outgrown their connective tissue. One aspect that could be related to this alteration is the thickness of the perimysium septa of the *Pectoralis major*. In fact, Ahn *et al.* (2010) observed that the connective tissue which covers the muscle is thinner in fast-growing chickens respect to the slow-growing counterpart and could be one of the reasons of this myopathy (Baldi *et al.*, 2018).

At the gross examination, this myopathy appears as loose of integrity of the muscle in the cranial portion of the *Pectoralis major*. At the touch the cranial part has softer and more friable consistency respect to normal tissue (Baldi *et al.*, 2018) (Figure 1.4).

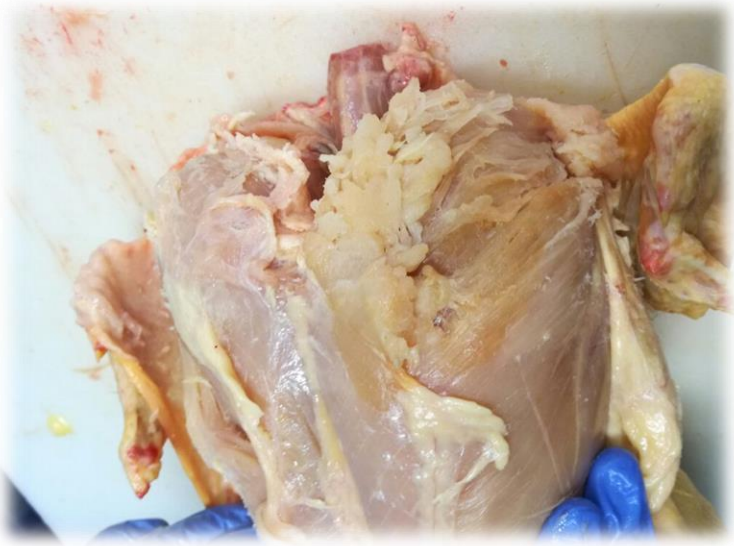

Figure 1.4 Image of severe Spaghetti meat.

At histological level, as for WS and WB, Spaghetti meat is characterized by extensive degenerative processes and regeneration, hyalinization, fibres with different structure, compromised connective tissue, abundant infiltration of inflammatory cells, presence of split fibres and lipidosis (Baldi *et al.*, 2018). Unlike WS and WB, a progressive rarefaction of the endomysium and perimysium has been observed leading to

detachment of muscle fibres. For all these reasons, usually this alteration does not appear alone but is associated with other myopathies.

Regarding the occurrence at commercial slaughterhouses, data are still sporadic, but the few available data reported that SM is going to be observed more and more frequently and affects especially females.

SM also affects the quality of the fillets; Baldi *et al.* (2018) detected that breast muscle affected by this alteration are heavier than normal fillets. As for WS, the principal alteration of meat quality seems to be the increase in meat ultimate pH without affecting other colorimetric parameters. Baldi *et al.* (2018) also observed that SM occurrence is associated with a remarkable decrease in the protein content and a relevant increase in moisture as for WB myopathy.

Factors affecting the occurrence of myopathies

The specific etiology of these alterations is still unknown but, in the years, numerous studies have been conducted to identify factors which could modify their occurrence or degree.

Genetics

The **genotype** of the broiler chicken is one of the most important factor affecting the occurrence of abnormalities linked to myodegeneration. Numerous studies demonstrated the relationship between growth rate and muscle alterations. Indeed, Petracci *et al.* (2015) reported that the muscles of animals belonging to the faster growth rate line, especially at level of breast muscle, are likely more subjected to unsustainable pressure on the metabolism of the breast muscle; this mechanism could lead to the development of degenerative features that are similar to muscular dystrophies. The increased hypertrophy of the fibres and the consequent increasing size of the muscle in animal of fast-growing genotypes could undermine the capillarization of the breast muscle causing negative effect on fiber metabolism and consequently pathological changes like myodegenerations associated with white striping and wooden breast. All these myopathic changes could be the follow up of a reduced oxygen supply to muscle as a result of lower capillarity density and decreased relative vascular support.

Lorenzi *et al.* (2014) described how under commercial conditions the incidence of WS on two different genotypes (medium *vs.* heavy) was significantly different. In fact, the author reported that the heavy flocks presented higher percentage of moderate and severe myopathy, whereas the incidence of moderate degree of WS was much higher than previous data published by other authors. This factor could be really important, because it could mean that the development of faster genetic lines might bring about the occurrence of breast alterations. However, other authors stated that genotype could be responsible for a minor contribution to the occurrence of these myopathies. Indeed, Bailey *et al.* (2015) confirmed that less than 35% of the variance in the occurrence of modern myopathies could be attributed to genotypes and the causes should be researched in environmental and management factors. However, recently other authors (Alnahhas *et al.*, 2014) highlighted the importance of genetics as major determinant of WS impacting on the muscle development. Indeed, these authors referred that some genotypes, characterized by heavier breast, are able to store energy as a glycogen and, in this way, more susceptible to WS.

B**ody weight** at slaughter seems to be another factor which plays an important role in the occurrence of abnormalities related to myodegeneration. Lorenzi *et al* (2014) observed a dramatic increase in the occurrence of WS in heavier chickens than medium-weight chickens. The severity of white striping also resulted different in the two classes of weight of animal. On the other hand, Kindlein *et al.* (2015) observed that heavier birds with thicker breasts were more subjected to wooden breast than lighter birds. No data is available about the effect of bodyweight on the incidence and severity of spaghetti meat.

The **growth rate** has been investigated to understand the relationship with the occurrence of the myopathies. Indeed, the authors agreed to affirm that the growth rate is a really important factor. As said above, the modern genetic lines are characterized by fast growth rate which is responsible for a rapid muscle development. Probably, the hypertrophy of the breast muscle, and thus, the increase in the diameter of the fibres of this muscle, is not supported by appropriate vessel supply or other supporting system. This theory has been supported by different author; in fact, Kuttappan *et al.* (2012) observed that broilers fed with different diet (low energy *vs.* high energy), at the same age, presented different body weight due to different growth rate and the fillets of the animals fed with high-energy diet were more affected by severe degree of WS than broilers fed with low-energetic diet. Similarly, Griffin *et al.* (2017) described how myopathies are linked with the growth rate. Therefore, nowadays, the control of growth rate of broiler chickens represents one of the most promising strategy to control the occurrence of these alterations.

The effect of **gender** on the occurrence of these alterations is not clear. Normally, the difference in the occurrence of meat alterations is related to the difference of live weights of the animals. Nevertheless, Lorenzi *et al.* (2014) reported that under commercial condition the incidence of WS was higher in males than in females at similar live weight. Kuttappan *et al.* (2013) found no significant effect of gender on WS occurrence, but they reported that females had a higher rate of normal breast whereas males showed a higher rate of severely WS breasts.

Feeding and nutrition

Different strategies to prevent the occurrence of these alterations have been studied in the last years. One of these is the inclusion in the diet of **tocopherol**. The myopathies previously described have shown lesions similar to the muscle dystrophy, which is a condition associated to a lack of vitamin E (tocopherol). Naturally, this vitamin is found in the plant and plays fundamental role as antioxidant. It helps to prevent the propagation of free radicals and, avoiding DNA damage, protects the fibres and muscle. The clinical manifestation of the lack of this vitamin includes macroscopic white striations in the breast muscle; these striations usually are oriented as the fibres of the muscle and sometimes can be observed also in the leg muscle. Also the histological features appear quite similar to WS. However, WS has been observed in animal fed with adequate level of tocopherol (Kuttappan *et al.,* 2012). Furthermore, no evidences have been reported to indicate a negative correlation between higher level of tocopherol and the occurrence of WS. Guetchom *et al.* (2012), after the inclusion in the diet of similar quantities of vitamin E, obtained similar live bodyweight in poultry fed the diets with and without tocopherol supplementation. However, they observed, at histological level, that at 28 days of age the broilers fed higher quantity of vitamin E were less affected by breast muscle damage. However, this positive effect disappeared at 42 d of age.

Selenium is another important supplement which may be used in broiler diets. It is a trace mineral fundamental, as tocopherol, in the immune response and acting like an antioxidant. For this function, it has been studied to understand if it could play a role in the control of myopathies, in particular WS and WB;

however, Ferreira *et al.*, 2016 did not observe significant effect of dietary Se on the occurrence of the two myopathies.

Another nutrient which could modify the occurrence of myopathies is **lysine,** an essential amino acid important for the correct growth of the animal. In a recent study, some authors (Cruz *et al.,* 2017) experienced two diets with different level of lysine. The birds fed with higher level of lysine at 35 days and 42 days of age presented higher bodyweight, breast and carcass weight and a higher occurrence of the two myopathies compared to the control group fed basal level of lysine. So, the higher incidence in the experimental group could be explained with the growth rate of the experimental groupps. Accordingly, Meloche *et al.* (2018) reduced the lysine dietary level during critical periods of the life and found a reduction in the occurrence and the severity of these myopathies.

Arginine is another amino acid likely implied in broiler myopathies. As said above, the lesions of these myopathies are quite similar to hypoxic injury. So, arginine, for its vasodilator activity, has been studied to understand if it was possible to improve blood flow to the breast muscle and, in this way, reduce the incidence of WS and WB. Another property of this amino acid is the fact that it can exercise an antioxidant activity. However, Christensen *et al.* (2016) reported that the inclusion in the diet of this amino acid did not impact on the severity of WS and WB.

The use of **anticoccidial** plays an indirect role in the incidence of breast muscle alteration. Indeed, the toxicity on skeletal muscles of these molecules has already been reported by different authors (Sandercock and Mitchell, 2003; Chapman *et al.*, 2010). In this contest, Dalle Zotte *et al.* (2015), observed that animals fed with a diet containing an anticoccidial additive were more affected by WS than animals vaccinated against coccidiosis. Also in this case, the effect was due to the different growth rate of the animals. Indeed, animals of the vaccinated group resulted to have lighter carcass respect to the animals fed with anticoccidial additive.

Thus, if reducing the growth rate is likely a strategy to control myopathy occurrence, **feed restriction** could be successfully used to this aim. As stated above, one of the most effective tools to control the incidence of the myopathies is acting on the growth rate of the animal. In this context, Kuttappan *et al.* (2013) succeeded in reducing growth rate, final live weight and breast weight, and thus, reducing the incidence of breast alterations in broilers reared for 54 days of age giving to the animals a diet characterized by low energy. Furthermore, a dietary restriction from 7 till 47 d of age, raising the feeders from 9 a.m. to 5 p.m., reduced live final weight, growth rate and, was successful at reducing the occurrence of myopathies (Livinston *et al.*, 2016).

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Aims

During the last years, the poultry industries aimed at increasing the production and standardizing the meat quality. To achieve these results the producers selected genotypes characterized by rapid growth rate, heavy live weights, and high breast yield. However, at the slaughterhouse, the occurrence of new abnormalities, in particular affecting the most valuable cut, the breast, increased more and more. The myopathies most commonly observed are white striping, wooden breast, and spaghetti meat, which could result in downgrading or, even, elimination of affected meat. The occurrence rate is quite variable and, according to literature, may range from 40% to 90% for white striping and 7% to 50% for wooden breast. No data are yet available for the occurrence of spaghetti meat.

At macroscopic examination, the affected breasts show white striations over the surface of the breast, and/or hard consistency and bulging on cranial and caudal part of the fillets, or loss of muscle fibres organization. At the macroscopic observations, these three pathologies appear quite different, whereas at histological level they share some common features. Indeed, the tissue affected show degenerative and necrotic phenomena, with lymphocyte and macrophage infiltration, various degree of fibrosis and lipidosis, and fibre regeneration.

The aetiology of myopathies is still under discussion and seems to be not related to only one cause. Some Authors suggest that the origin could be a failure in blood supply: the rapid and great increase in *P. major* muscle size is not supported by a corresponding increase of the bloody vessels in muscle fibres resulting into a poor oxygenation of the tissue and in degenerative changes. Another consequence of the impaired growth of the bloody vessels is the displacement of metabolic waste products. All these changes resulted in and excessive production and release of reactive oxygen species, which might be responsible of the initial inflammatory phase of these abnormalities.

Thus, during these years, literature has shown that the occurrence of myopathies is related to:

- genotypes, selection for rapid growth rate or high breast yield results in high myopathy rates;
- growth rate, excessive growth rates are associated to increased myopathies rates;
- live weight and breasts weight, a positive correlation between high final live and breast weight and myopathies rates and degree have been measured;
- gender, some abnormalities occur more in one gender compared to the other.

Basing on these evidences, one strategy to control the occurrence and severity of these alterations would be the limitation of bird growth rate. These results could be achieved by different means, out of which feed restriction appears to be one of the most promising solution. On the other hand, it could also be a great threat to the poultry industry, because of consequences on final weight and productive performances, thus resulting in a loss for the producer.

Thus, investigation is required to define the best management of feed restriction to reduce growth rate, but to maintain global productive performances.

Accordingly, the aim of this PhD Thesis was to investigate the effect of different strategies to control growth rate by feed intake and of different ontogenetic factors on performance and occurrence myopathies. In details, the following feeding strategies and ontogenic factors were evaluated:

- *ad libitum* feeding *vs* early feed restriction (13 to 21 d) (trial 1);
- *ad libitum* feeding *vs.* early restriction (13 to 23 d) *vs.* late restriction (27 to 37 d) (trial 2);
- reduction of feeding hours by means of light program (14 *vs.* 18 hours of light) (trial 3);
- effect of gender (trial 1, 3);
- effect of genotypes (trial 1, 2, 3).

Moreover, the thesis investigated about:

- the effect of myopathies (type and severity) on meat quality (trial 3, 4);
- the rheological and microbial shelf-life of affected meat during storage (trial 4).

CHAPTER 2: FIRST CONTRIBUTION

Effect of early feed restriction on growth, carcass and meat quality and muscle fibre degeneration associated with myopathies in broiler chickens

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ABSTRACT

To evaluate the effect of genotype (standard breast yield *vs.* high breast yield), gender, and feeding systems (*ad libitum vs.* feed restriction from 13 to 21 d of age) on slaughter results and meat quality 768 broiler were evaluated; moreover to investigate muscle fibre degeneration (MFD) associated with white striping and wooden breast, 192 *Pectoralis major* (4 animals for experimental group) were sampled at 14, 21, 28, 35, and 46 d of age for histological analyses by hematoxylin and eosin (H&E) staining to evaluate tissue morphology, Masson's trichrome to identify collagen presence, and Oil red and Nile blue for lipid presence. Slaughter results and meat quality were evaluated in 768 broilers according to genotype. Standard-yield chickens had higher carcass weights (2358 g *vs.* 2319 g; $P < 0.001$) and hind legs proportion (31.1% *vs.* 30.6%; $P < 0.01$), and lower dressing out percentage (73.6% *vs.* 74.0%; $P < 0.01$) compared to high-yield birds, besides lower meat L* index (45.3 *vs.* 46.2; $P < 0.05$), higher final pH (5.89 *vs.* 5.85; $P < 0.05$) and thawing losses (10.5% *vs.* 9.43%; $P < 0.05$). Males showed higher carcass weight (+24%), dressing percentage (+0.7%), and hind leg yield (+4%) ($P < 0.001$) than females. Restricted birds had lower carcass weight (–2%; $P < 0.001$) and dressing percentage (-0.3%) ($P < 0.05$) than those always fed *ad libitum*. As what concerns meat quality, gender and feeding system affected only meat final pH, lower in *ad libitum* group than in restricted one and in females than males. Microvessels (diameter ≤15 μm), nuclei positive to anti-cleaved lamin A and monoclonal proliferating cell nuclear antigen (PCNA) antisera were counted to assess apoptotic and regenerative processes, respectively. Significant differences were found according to feeding system, age, and their interactions. The frequency of chickens with MFD was higher with ad libitum than restricted feeding (75.0% *vs.* 62.5%; $P = 0.01$) and increased with age (18.8%, 28.1%, 75.1%, 96.9%, and 96.9% at 14, 21, 28, 35, and 46 d). However, at 14 d a similar frequency (18.8%) was found in all broilers; at 21 d, MFD occurred more in broilers fed ad libitum than in those under restriction (50.0% *vs.* 6.3%; $P < 0.01$); at 28 d differences were reduced (87.5% *vs.* 62.5%; $P = 0.10$) to disappear by 35 (100% and 93.8%) and 46 d (96.9% and 96.9%). The number of microvessels decreased with age (20.7 to 9.46; $P < 0.001$) and the number of nuclei positive to the anti-cleaved lamin A antibody increased. At histology, MFD at 46 d corresponded to loss of typical cross striations, massive necrotic process, degenerating fibres surrounded by inflammatory cells, scattered fibres in an abundant collagen-rich connective tissue, numerous adipose cells; necrotic fibres showed a high percentage of apoptotic nuclei, and regenerating fibres appeared positive to anti-PCNA antibody. In conclusion, slaughter results and carcass traits changed especially with genotype and gender, coherently with slaughter weight whereas meat quality was mostly affected by genotype; MFD soon occurred after 2 weeks of growth and increased dramatically within 28 d. Early feed restriction reduced MFD as long as animals were restricted, but no residual effect was recorded after re-alimentation.

INTRODUCTION

The high productivity of poultry is the result of an intense genetic selection for fast growth rate and high breast yield, which has been continuously carried out during the last decades. The animals selected have to assure profitable performance and constant traits and meat quality (Petracci *et al.*, 2015). However, this selection led to the occurrence of morphological abnormalities in skeletal muscles, large fiber diameters, as well as high proportion of glycolytic fibres (Dransfield and Sosnicki, 1999). Firstly, genetic selection for growth was associated with muscular problems, such as leg weakness and edema, focal myopathy, deep pectoral myopathy, and muscular dystrophy in broilers and turkeys (Siller, 1985). More recently, high growth rate and high breast yield of the most common genetic lines have been associated with the occurrence of other myopathies affecting pectoralis major and other muscles, i.e., white striping (WS) and wooden breast (WB) (Kuttappan *et al.*, 2012a, 2013a; Lorenzi *et al.*, 2014), and thus affecting the rheological and nutritional traits of poultry meat. In presence of WS, pectoralis major exhibits a degenerative myopathy at fiber level (Kuttappan *et al.*, 2013b), higher final pH, some minor differences in color indexes lightness, red, and yellow), and higher cooking losses compared to not affected muscles (Mudalal *et al.*, 2014; Mazzoni *et al.*, 2015; Trocino *et al.*, 2015). In case of WB, pectoralis major also shows polyphasic myodegeneration with regeneration and accumulation of interstitial connective tissue or fibrosis at histology (Sihvo *et al.*, 2014; Velleman and Clark, 2015), besides higher water losses during cooking and shear force compared to normal breasts (Mudalal *et al.*, 2014; Trocino *et al.*, 2015). Indeed, low and similar patterns of heritability and genetic correlations for breast meat abnormalities (white striping, wooden breast and deep pectoral myopathy) have been measured in 2 genetic lines with different breast yields (Bailey *et al.*, 2015). In addition, literature reports a wide variability at slaughter in myopathy occurrence (12% to > 70% at gross examination) which has been partially attributed to differences in bird live weight (Kuttappan *et al.*, 2013a; Petracci *et al.*, 2013b; Lorenzi *et al.*, 2014; Trocino *et al.*, 2015). Accordingly, all factors affecting growth rate of broilers could play a role in modifying the occurrence of myopathies, e.g. feeding regime (Kuttappan *et al.*, 2012; Trocino *et al.*, 2015) or gender (Trocino *et al.*, 2015). In this contest, the use of particular feeding strategies, like feed restriction may control growth and health, but it may affect carcass and meat quality (Sahraei, 2012; Butzen *et al.*, 2013). To our knowledge, no information is available on the onset of myopathies according to age and/or live weight. For this reason, the present study aimed at evaluating the histological and immunohistochemical changes at different ages (14, 21, 28, 35, and 46 d) associated with the occurrence of myopathies in the pectoralis major muscle fibres of chickens belonging to 2 genetic lines (selected for standard or high breast yield), of both genders, fed *ad libitum* or at a restricted rate during the first growth period (from 13 to 21 d of age).

MATERIALS AND METHODS

Experimental facilities

The trial was performed at the poultry house of the Experimental Farm "Toniolo" of the University of Padova (Legnaro, Padova, Italy) during June and July, after a long period of downtime (<6 months). The poultry house was equipped with cooling system, forced ventilation, radiant heating and controlled light systems. Thirty-two wire-net pens (125 cm wide x 177 cm large x 120 cm height; 2.2 m^2) were available, each equipped with an automatic circular drinker (diameter: 39 cm) and a circular feeder (diameter: 37 cm) for manual distribution of feed. The pens had a concrete floor bedded by wood shavings litter (height 5 cm, 2.5 kg/m^2). Before chicks arrival, farm environmental temperature was raised to about 26°C. Infrared lights were used in pens at the level of the chicks during the first days. Supplemental manual drinkers and feeders were used until chicks were able to reach the circular drinkers and feeders. During the trial, environmental conditions were monitored daily (maximum temperature: 28.5±2.1°C; maximum humidity: 70.5±6.4%).

Twenty-four hours of light were provided during the first two days after chicks arrival; afterward, hours of lights were progressively reduced to reach and maintain a 18L:6D light program from the 12^{th} day onwards.

Animals and experimental groups

A total of 896 chicks were delivered by authorized transport truck at the experimental facilities of the University on the hatching day. Half of the chicks belonged to a high-breast-yield hybrid, the other half were from a standard-breast-yield genotype and both were sexed at the hatchery. Birds belonging to the two genotypes were obtained from two different hatcheries and were housed after 2.5 h and 45 min of transport, respectively. All chicks had been vaccinated against Marek's disease, Infectious Bronchitis, and Newcastle disease at the hatchery.

At their arrival, 28 chicks per pen were housed, randomly allocated to 8 experimental groups, *i.e.* 2 genotypes x 2 genders x 2 feeding plans (*ad libitum vs.* restricted), and controlled from the day after their arrival until slaughtering at 46 d of age.

At 14, 21, 28 and 35 d of age and at live weight equal to 547±50 g, 1013±97 g, 1770±192 g, and 2380±258 g, respectively, 32 chickens per each age (one chicken per pen) were slaughtered by cervical dislocation to sample muscles for histological analyses. At 46 d of age, among chickens submitted to commercial slaughter, 64 animals (2 birds per pen) (3197±342 g) were selected as representative in terms of average live weight and variability of the corresponding pens and used to sample *p. major*.

Diets and feeding plans

Four commercial diets were administered during the trial, *i.e.* diet P1 (crude protein 22.2%, ether extract 7.90%; crude fiber 2.60%, calcium 1.00%, and phosphorus 0.70%) from 0 to 12 d, diet P2 (crude protein 20.8%, ether extract 8.50%; crude fiber 2.50%, calcium 1.00%, and phosphorus 0.65%) from 13 to 21 d, diet P3 (crude protein 19.0%, ether extract 8.10%; crude fiber 2.50%, calcium 0.95%, and phosphorus 0.60%) from 22 to 35 d and diet P4 (crude protein 17.4%, ether extract 8.80%; crude fiber 2.40%, calcium 0.80%, and phosphorus 0.60%) from 35 d until slaughtering. Diets were produced by a commercial feed mill (Mangimificio Settecolli, Montegalda, Vicenza, Italy).

Chickens from half pens were fed *ad libitum* during the experimental trial, the remaining ones were restricted in the period 13-21 d of age. The restricted birds received the 80% of the quantity consumed by the chickens fed *ad libitum* on the previous day. The restriction program was calculated separately on the four groups obtained by the combination of 2 genotypes x 2 genders.

Growth performance and health status

Chicks were individually weighed the day after their arrival, identified by a leg mark, and controlled for live weight once a week until slaughtering. Pen feed consumption was measured daily during the trial. Health status of birds was checked daily by clinical examination. *Post-mortem* examination of dead birds was performed to evaluate the presence and the type of pathological change. A total of 5.2% losses were recorded during the trial of which 3.5% due to mortality and 1.7% due to birds culled before slaughtering because of lameness.

Commercial slaughtering and carcass and meat quality recordings

At 46 d of age, all birds were slaughtered in a commercial slaughterhouse, after about 7 hours of feed withdrawal and about 4 hours of drinking withdrawal. Birds were individually weighed before crating. All birds of a pen (i.e. 20 to 24) were loaded in a transport cage (62.6 cm wide x 160 cm long x 25.0 cm high; 1 m^2). Loading took about one hour; transport from the experimental facilities to the commercial slaughterhouse about 15 min; lairage before slaughtering about 3 hours. Birds were slaughtered according to the usual practice of the commercial slaughterhouse. Eviscerated carcasses without feathers, head, neck, abdominal fat, and feet were recovered after 2 hours of refrigeration at 2°C and individually weighed to measure slaughter dressing percentage (World's Poultry Science Association, 1984).

A total of 256 carcasses (8 per pen) had been previously selected on the basis of the slaughter live weight to be representative within a pen and to be submitted to gross examination to evaluate the occurrence and the

degree of white striping on *pectoralis major* muscle (Kuttappan *et al.*, 2012b), and the occurrence of wooden breast (Sihvo *et al.*, 2014) which results have been presented by Trocino *et al.* (2015).

Afterwards, out of the 256 carcasses, 128 specimens (4 per pen) were further selected, as representative of the average live weight and variability of each pen, and transported to the Department laboratories to be stored at 2°C before carcass and meat quality analyses. Forty-eight hours after slaughter, carcasses were dissected for the main cuts (breast, wings, thighs, and drumstick) (World's Poultry Science Association, 1984).

Meat and bones were separated from drumstick. *Pectoralis major* muscles were then separated from the breasts to be submitted to meat quality analyses (Petracci and Baéza, 2011). The pH of the *P. major* muscles was measured in triplicate on their ventral side with a pH meter (Basic 20, Crison Instruments Sa, Carpi, Italy) equipped with a specific electrode (cat. 5232, Crison Instruments Sa, Carpi, Italy). The L*a*b* color indexes were measured in triplicate in the ventral side of the same muscles covered by a transparent plastic film, using a Minolta CM-508 C spectrophotometer (Minolta Corp., Ramsey, NJ, USA) (Petracci and Baéza, 2011).

Thereafter, one parallelepiped meat portion (8 cm x 4 cm x 3 cm) was separated from the cranial side of *P. major*, parallel to muscle fibres directions, and stored under vacuum in plastic bags at -18°C until the meat analyses. Thawing and cooking losses were measured in this cut (Petracci and Baéza, 2011). After thawing, the meat portion was put in plastic bags and cooked in a water bath for 45 minutes, until an internal temperature of 80°C was achieved. After a 40-minutes cooling, a further parallelepiped meat portion (4 cm x 2 cm x 1 cm) was separated from the original parallelepiped. On this latter section, the maximum shear force was measured with LS5 dynamometer (Lloyd Instruments Ltd, Bognor Regis, UK) using the Allo-Kramer (10 blades) probe (load cell: 500 kg; distance between the blades: 5 mm; thickness: 2 mm; cutting speed: 250 mm/min) (Mudalal *et al.*, 2014).

Histological analysis

At each intermediate slaughter, *P. major* muscles were immediately sampled for histology. At final slaughter, 46 d, 64 out of the 128 chickens to be dissected, representative of the average live weight and variability of each pen, were immediately used to sample *P. major* and *P. minor* muscles for histological analysis.

Samples were fixed in 10% buffered neutral formalin at 4°C overnight, washed in phosphate-buffered saline (PBS, 0.1 M, pH 7.4), dehydrated through a graded series of ethanol and embedded in paraffin. Samples to be submitted to histochemistry were frozen in isopentane cooled by liquid nitrogen.

Sections were cut at a thickness of 4 μm using a microtome (cryostat for frozen samples) and stained with: i) hematoxylin and eosin (H&E), ii) Masson's Trichrome, iii) Oil red, iv) Nile blue. H&E staining was

employed to evaluate the general morphology of the tissues, Masson's Trichrome (Bancroft and Stevens, 1975) was used to qualitatively assess the amount of collagen, whereas Oil red and Nile blue (Bancroft and Stevens, 1975) were used to identify the presence of lipids.

At microscopy examination (Olympus Vanox photomicroscope, Japan), myopathic lesions, lipidosis and fibrosis were assessed using a score ranging from 0 to 3 (0, normal; 1, mild; 2, moderate; 3, severe). In details, the score (0) was attributed to samples presenting no necrotic fibres, no infiltration of connective tissue, and with normal or central nuclei; the score (1) was used when samples showed central nuclei, some fibres with hyaline cytoplasm, scarce necrotic fibres, absence of connective tissue infiltration; the score (2) was given when samples diffusely presented necrotic fibres, thickening of interstitial connective tissue, presence of inflammatory cells, and appearance of adipose tissue aggregates; finally, the highest score (3) was attributed to samples exhibiting a great amount of interstitial connective tissue and inflammatory cells, as well as of necrotic fibres and lobules of adipose tissue.

In addition, microvessels (diameter $\leq$ 15 μm) were counted on histological sections (Olympus Vanox photomicroscope, Japan) of *P. major* muscles of all chickens slaughtered at 14 d and 46 d of age. Within each section, microvessels were counted independently in five areas (60.000 μm^2 each) by two trained observers.

Immunohistochemistry was carried by an automated immunostainer (BenchMark Ultra, F. Hoffmann-La Roche AG, Basel, Switzerland). Sections were deparaffinised in xylene, rehydrated in graded ethanol and rinsed in distilled water. Heat-induced antigen retrieval was performed using the benchmark ULTRA Cell Conditioning Solution CC2 (Ventana Medical Systems, Inc., Tucson, AZ, USA) (pH=6.0) at 91°C for 44 min. Sections were incubated for 32 min at room temperature with the primary polyclonal antibody anti-cleaved lamin A (cod. 2035, Cell Signaling Technology, Danvers, MA, USA) diluted 1:100. Sections were then incubated with the detection system UltraView Universal DAB detection kit with HRP (horseradish peroxidase) enzyme directly conjugated to the secondary antibody (Hoffmann-La Roche AG, Basel, Switzerland). The quantitative assessment of anti-cleaved lamin A positive nuclei was made to assess the apoptotic processes by using a computerized image analyser system (Olympus CellB, Japan) and as follows: (1) each haul was represented by three sections from each muscle sample; (2) three fields from each muscle section were analysed; (3) within each field (60,000 μm^2), the number of positive nuclei was measured.

Three serial sections per each slaughtering were manually immunostained with a mouse monoclonal PCNA antiserum diluted 1:500 (Cell Signaling Technology, Danvers, MA) and incubated overnight at +4°C with the detection system EnVision FLEX/HRP and the EnVision FLEX Substrate Buffer EnVision FLEX DAB (Agilent Technologies, Dako Denmark A/S, Glostrup, Denmark). Then EnVision FLEX Substrate Buffer EnVision FLEX DAB was used as chromogen and all sections were counterstained with the EnVision FLEX Hematoxylin to assess the regenerative processes. The specificity of the immunostaining was verified by incubating sections with: (1) PBS instead of the specific primary antibodies; (2) preimmune sera instead of

the primary antisera; (3) PBS instead of the secondary antibodies. The results of these controls were negative (i.e. staining was abolished). Moreover, skin and thymus of chickens were used as positive control tissues for both primary antibodies.

Statistical analysis

Individual data of initial and final weights, daily growth, slaughter results, and carcass and meat traits were analysed by ANOVA with feeding system, genotype, gender, and their interaction as main factors of variability and with pen as a random effect, by the PROC MIXED of SAS software (SAS Institute Inc., 2011). Cage data for feed intake and feed conversion were analysed by ANOVA with feeding system, genotype, gender, and their interactions as main factors of variability, and by the PROC GLM (SAS Institute Inc., 2011). When necessary, the Bonferroni *t* test was used to compare least squares means. The frequency of chickens showing any degree of muscle fibre degeneration (MFD) at *P. major* was analysed by PROC CATMOD (SAS Institute Inc., 2009) according to genotype, gender, feeding system, and age. Thereafter, differences according to the feeding system within age were assessed by the χ^2 test. The scores for muscle fibre degeneration, the number of apoptotic nuclei and the number of microvessels were analysed by PROC GLIMMIX (SAS Institute Inc., 2009) with genotype, gender, feeding system, and age and their interactions as fixed effects.

Differences between the means with $0.05 < P \leq 0.10$ were accepted as close to statistical significance.

RESULTS

Growth performance

The live weight of the broilers belonging to standard yield genotype was lower from the beginning of the trial ($P < 0.001$), similar on day 22 and, at the end of the trial, higher for the high-breast yield chicks, due major daily weight gain (+3.6%; $P < 0.001$) in the second period of growth. Feed conversion was significantly different in the two strains; standard genotype had lower conversion during both the periods: in the first period due to the significantly lower feed intake, -2.4%; $P < 0.01$ and in the second period mostly due to the significantly higher daily weight gain, resulting thus in a 3% improvement on the whole trial ($P < 0.001$) (Table 2.1).

At the arrive the two genders had the same weight; however, at 22 d of age male and female had significantly different weight ($P < 0.001$). During the whole trial, male showed higher daily weight gain (+23%) and feed intake (+17%) and lower feed conversion (-6%) (Table 2.1).

The application of feed restriction affected coherently the performances of the broilers. The early restriction, from 13 to 22 d of age impaired the live weight of the animals and a difference was observed till the end of the trial, whereas it has been reduced. Indeed, at 22 d *ad libitum* group was heavier than restricted one (+15%; $P < 0.001$) because of the higher feed intake (+16%; $P < 0.001$) and daily weight gain (+15%; $P < 0.001$). On the other hand, at the end of the trial, the compensatory growth of the restricted animals (+4%; $P < 0.001$) achieved to reduce the difference in live weight of the two groups (-2%; $P < 0.01$). Accordingly, feed restriction improved feed conversion during the second period (3%; $P < 0.001$) and the whole trial (2%; $P < 0.01$).

The genotype and the gender showed some significant interactions (Figure 2.1). Indeed, the difference in the final live weight in males and females belonging to high-breast-yield genotype was higher (+26%) than the difference between males and females of the other genotype (+20%) ($P < 0.001$) corresponding to daily weight gain ($P < 0.001$) and feed intake ($P < 0.05$) during the second period of growth (Figure 2.1).

Also, significant interactions were measured for the feeding systems and the genotypes of birds during the second period: the lowest daily weight gain was measured in the high-breast-yield chickens always fed *ad libitum*, the highest daily weight gain was recorded in birds of the standard genotype previously submitted to feed restriction, whereas intermediate values were measured in the other two experimental groups ($P < 0.05$) (Figure 1). Feed intake changed with a similar trend (Table 2.1).

Table 2.1. Productive performance[1] (LS means±SEM) of broiler chickens until slaughtering

	Breast yield (B)		Gender (G)		Feeding system (F)		*P*-value						
Item	Standard	High	Females	Males	*ad libitum*	Restricted	B	G	F	F×B	F×G	B×G	F×B×G
Chickens (n)	364	364	363	365	362	366							
Live weight (g)													
On day 1	50±0.37	53±0.39	51±0.37	52±0.39	51±0.38	51±0.38	<0.001	0.32	0.57	0.37	0.97	0.79	0.95
On day 22	952±7.68	949±8.15	887±7.71	1014±8.11	1023±7.96	878±7.87	0.73	<0.001	<0.001	0.61	0.15	0.61	0.61
On day 46	3,207±14.0	3,130±14.3	2,845±13.9	3,492±14.4	3,194±14.2	3,142±14.1	<0.001	<0.001	<0.01	0.13	0.24	<0.001	0.76
First period (1-22 d)													
Weight gain (g/d)	43.0±0.36	42.7±0.38	39.8±0.36	45.9±0.38	46.3±0.37	39.4±0.37	0.43	<0.001	<0.001	0.57	0.15	0.61	0.61
Feed intake (g/d)	56.3±0.35	57.7±0.35	54.2±0.35	59.8±0.35	61.5±0.35	52.5±0.35	<0.01	<0.001	<0.001	0.91	0.09	0.62	0.63
Feed conversion	1.31±0.01	1.34±0.01	1.35±0.01	1.30±0.01	1.33±0.01	1.33±0.01	0.03	<0.001	0.90	0.62	0.55	0.83	0.92
Second period (23-46 d)													
Weight gain (g/d)	95.1±0.43	91.8±0.43	82.4±0.42	104.5±0.44	91.5±0.43	95.4±0.43	<0.001	<0.001	<0.001	0.04	0.50	<0.001	0.88
Feed intake[2] (g/d)	166±0.90	168±0.90	153±0.90	181±0.90	166±0.90	168±0.90	0.81	<0.001	0.14	0.73	0.37	0.02	0.94
Feed conversion[3]	1.76±0.01	1.83±0.01	1.85±0.01	1.74±0.01	1.82±0.01	1.76±0.01	<0.001	<0.001	<0.001	0.02	0.52	<0.01	0.74
Whole trial (1-46 d)													
Weight gain (g/d)	71.0±0.29	69.2±0.29	62.8±0.28	77.3±0.29	70.7±0.29	69.5±0.29	<0.001	<0.001	<0.01	0.13	0.24	<0.001	0.76
Feed intake[4] (g/d)	110±0.56	111±0.56	102±0.56	119±0.56	113±0.56	109±0.56	0.29	<0.001	<0.001	0.65	0.17	0.02	0.86
Feed conversion	1.56±0.01	1.61±0.01	1.63±0.01	1.54±0.01	1.60±0.01	1.57±0.01	<0.001	<0.001	<0.01	0.06	0.99	0.06	0.98

[1]Individual data: live weight and daily growth rate. Pen data: feed intake and feed conversion.
[2]Interaction Breast yield x Gender, $P < 0.001$: 154 and 179 g/d in standard yield females and males, and 151 and 182 g/d in high yield females and males.
[3]Interaction Breast yield x Feeding system, $P < 0.05$: 1.77 and 1.75 in standard yield chicks fed *ad libitum* and restricted, and 1.87 and 1.78 in high yield chicks fed *ad libitum* and restricted. Interaction Breast yield x Gender, $P < 0.01$: 1.79 and 1.72 in standard yield females and males, and 1.90 and 1.75 in high yield females and males.
[4]Interaction Breast yield x Gender, $P < 0.05$: 103 and 117 g/d in standard yield females and males, and 102 and 120 g/d in high yield females and males.

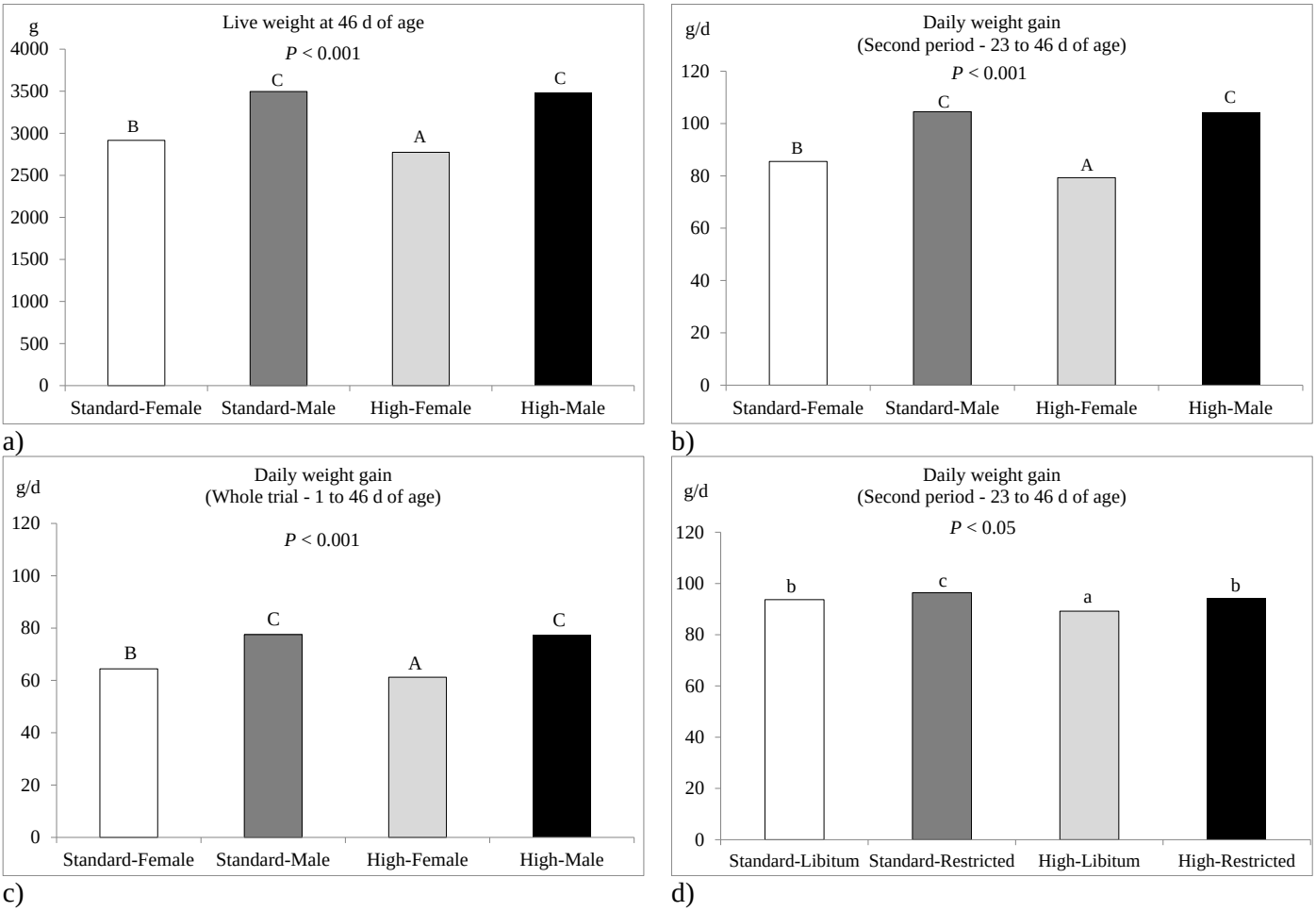

Figure 2.1. Live weight at 46 d (a) and daily weight gain during the second period of the trial (b) and during the whole trial (c) in female and male chickens of the standard and high-breast yield genotype (significant interaction genotype x gender); daily weight gain during the second period (d) in chickens of the standard and high-breast yield fed *ad libitum* or submitted to feed restriction (significant interaction genotype x feeding system). Means with different superscript letters significantly differ.

Slaughter results and carcass traits

At slaughter, the chickens of the standard genotype were heavier ($P < 0.001$) and had higher carcass weights ($P < 0.001$) and dressing out percentage ($P < 0.01$) compared to the chickens of the high-breast-yield genotype (Table 2.2). Breast yield did not vary with the genotype, whereas the thighs yield was higher in the standard genotype compared to the high-breast-yield one (+3.3%; $P < 0.01$) (Table 2.2).

The significant advantage of males *vs.* females in terms of live weight was confirmed on carcass weight and dressing percentage at commercial slaughter ($P < 0.001$) (Table 2.2). Males showed a higher yield in both thighs and drumsticks, which resulted in a higher yield in hind legs (+4%; $P < 0.001$) compared to females ($P < 0.001$) (Table 2.2).

The impairment of live weight in chickens submitted to restriction compared to those fed *ad libitum* was still evident at slaughter in terms of lower carcass weight, dressing percentage (-0.3%; $P < 0.05$), breast yield (-2.8%; $P < 0.10$), and thighs yield (-3.3%; $P < 0.05$) (Table 2.2).

Quality traits and occurrence of myopathies in *P. major*

The effect of genotype on meat quality was expressed in terms of lower L* index (-2%; $P < 0.05$), higher final pH (+0.7%; $P < 0.05$) and thawing losses (+11%; $P < 0.05$) measured on the *P. major* muscle of the chickens of the standard genotype compared to those of the high-breast-yield genotype (Table 2.3).

The feeding system only affected the *P. major* final pH, which was 0.7% lower in the chickens fed *ad libitum* compared to those submitted to feed restriction ($P < 0.01$). The difference in the value of the L* index according to the feeding system (+1.5% in chickens fed *ad libitum*) only approached significance ($P = 0.10$) (Table 2.3). The occurrence of white striping was lower in chickens always fed *ad libitum* (-10 percentage units) at a level approaching significance ($P = 0.07$) (Figure 2.2). No difference was recorded in the degree of white striping (Figure 3) or in the occurrence of wooden breast (Figure 2.2) according to the feeding regime.

Table 2.2. Slaughter results and carcass traits (LS means±SEM) in chickens slaughtered at 46 d

	Breast yield (B)		Gender (G)		Feeding system (F)		*P*-value						
Item	Standard	High	Females	Males	*Ad libitum*	Restricted	B	G	F	F×B	F×G	B×G	F×B×G
Chickens, n	364	364	363	365	362	366							
Cold carcasses (CC), g	2,358±13	2,319±14	2,086±13	2,581±14	2,357±13	2,310±13	<0.001	<0.001	<0.001	0.23	0.18	<0.01	0.45
Dressing out percentage, %	73.6±0.11	74.0±0.12	73.5±0.11	74.0±0.12	73.9±0.12	73.7±0.11	<0.01	<0.001	0.02	0.36	0.57	0.02	0.10
Dissected carcasses, n	64	64	64	64	64	64							
Breast yield, % CC	39.9±0.57	40.1±0.61	40.3±0.58	39.7±0.60	40.6±0.59	39.5±0.59	0.68	0.34	0.06	0.23	0.31	0.24	0.29
Thighs, % CC	18.3±0.21	17.7±0.21	17.6±0.21	18.4±0.21	17.7±0.21	18.3±0.21	<0.01	<0.01	0.02	0.73	0.14	0.71	0.81
Drumsticks, % CC	12.7±0.11	12.8±0.11	12.6±0.11	13.0±0.11	12.9±0.11	12.7±0.11	0.53	<0.01	0.22	0.56	0.09	0.26	0.24
Hind legs, % CC	31.0±0.21	30.5±0.21	30.2±0.21	31.4±0.21	30.6±0.21	31.0±0.21	0.10	<0.001	0.25	0.96	0.81	0.36	0.40
Wings, % CC	9.7±0.10	9.5±0.10	9.5±0.10	9.7±0.10	9.6±0.10	9.6±0.10	0.11	0.07	0.80	0.06	0.50	0.91	0.35

Table 2.3. Rheological traits (LS means±SEM) of *Pectoralis major* muscle in chickens slaughtered at 46 d of age

	Breast yield (B)		Gender (G)		Feeding system (F)		*P*-value						
Item	Standard	High	Females	Males	*Ad libitum*	Restricted	B	G	F	F×B	F×G	B×G	F×B×G
Carcasses, n	64	64	64	64	64	64							
pH	5.89±0.02	5.85±0.02	5.85±0.02	5.89±0.02	5.85±0.02	5.89±0.02	0.04	<0.01	<0.01	0.07	0.02	0.61	0.14
L*	45.3±0.31	46.2±0.31	45.6±0.31	45.9±0.31	46.1±0.31	45.4±0.31	0.02	0.43	0.10	0.63	0.36	0.06	<0.01
a*	-0.70±0.09	-0.84±0.10	-0.79±0.09	-0.76±0.10	-0.78±0.10	-0.77±0.10	0.13	0.69	0.92	0.86	0.60	0.84	0.10
b*	14.0±0.33	13.6±0.35	14.2±0.34	13.4±0.35	14.0±0.34	13.6±0.34	0.16	0.02	0.30	0.27	0.75	0.05	0.45
Thawing losses, %	10.5±0.53	9.43±0.57	10.3±0.54	9.64±0.56	10.3±0.55	9.66±0.55	0.04	0.20	0.24	0.07	0.35	0.17	0.69
Cooking losses, %	22.7±0.48	23.7±0.48	22.7±0.48	23.8±0.48	23.0±0.48	23.4±0.48	0.15	0.09	0.51	0.58	0.24	0.55	0.60
Shear force, kg/g	3.03±0.17	2.98±0.17	2.80±0.17	3.21±0.17	2.98±0.17	3.02±0.17	0.83	0.08	0.86	0.42	0.44	0.45	0.37

Muscle fibre degeneration (MFD)

The percentage of chickens showing any degree of MFD at histological examination was 68.8% on average (all slaughtering) and was not affected by the genotype or the gender (Figure 2.4a, 2.4b), whereas significant effects of the feeding system and the chicken age were measured (Figure 2.4c, 2.4d). The frequency of birds showing MFD was higher in birds always fed *ad libitum* compared to those submitted to early feed restriction (75.0% *vs.* 62.5%; $P = 0.01$) (Figure 1c).

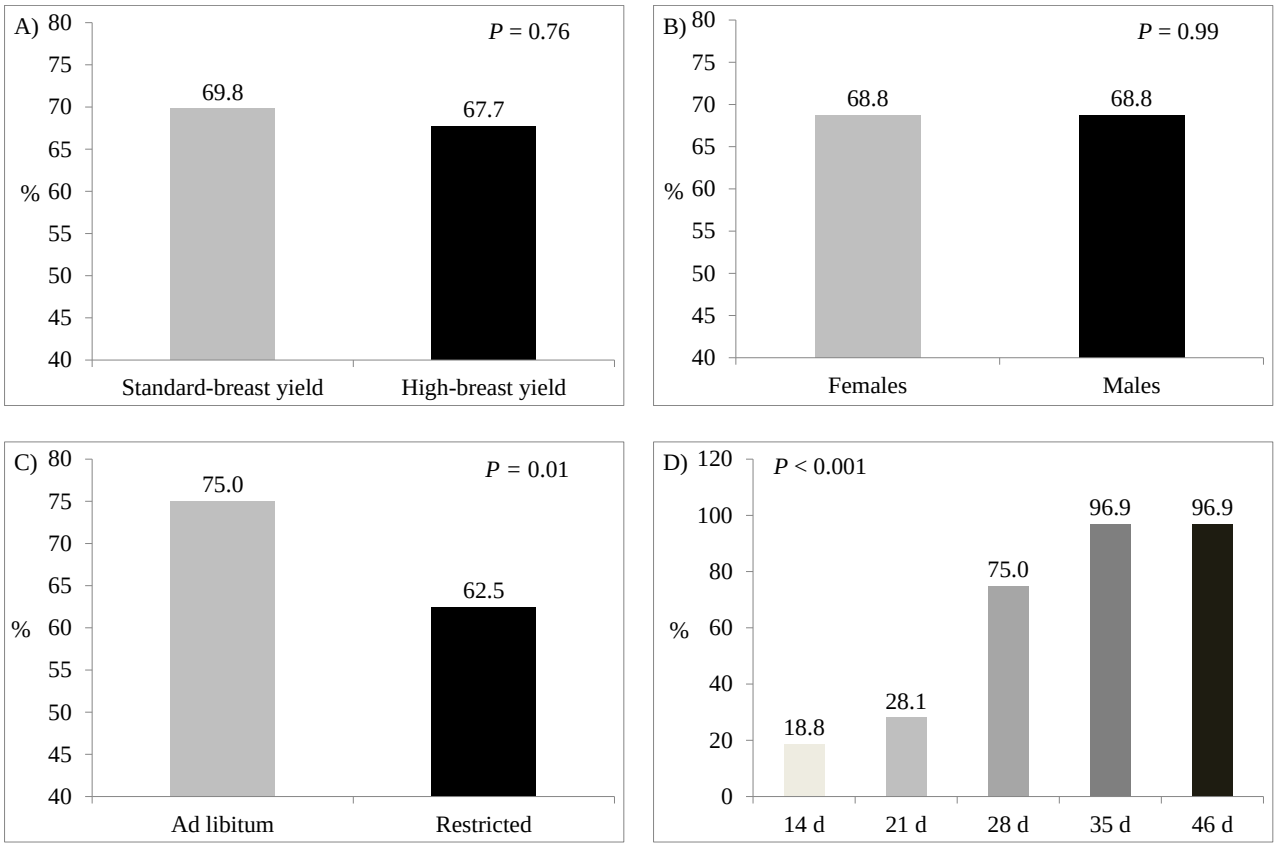

Figure 2.4. Percentage (%) of chickens showing muscle fibre degeneration at histological examination of *P. major* according to (A) genotype (standard *vs.* high-breast yield), (B) gender (females *vs.* males), (C) feeding regime (*ad libitum vs.* restricted from 13 d to 21 d of age), and (D) age of chickens (14, 21, 28, 35, and 46 d).

Moreover, as the age increased from 14 d to 46 d, the percentage of chickens showing MFD at *P. major* significantly increased ($P < 0.001$) (Figure 2.4d). Indeed, when the effect of the feeding system was evaluated within age (Figure 2.5), at 14 d of age a similar MFD occurrence was observed in all broilers; at 21 d (at the end of feed restriction) the MFD frequency was much higher in chickens fed *ad libitum* compared to restricted birds (50.0% *vs.* 6.3%; $P < 0.01$); at 28 d a similar trend was observed but differences between the two groups were reduced (87.5% *vs.* 62.5%, $P = 0.10$) to disappear within 35 d and 46 d of age.

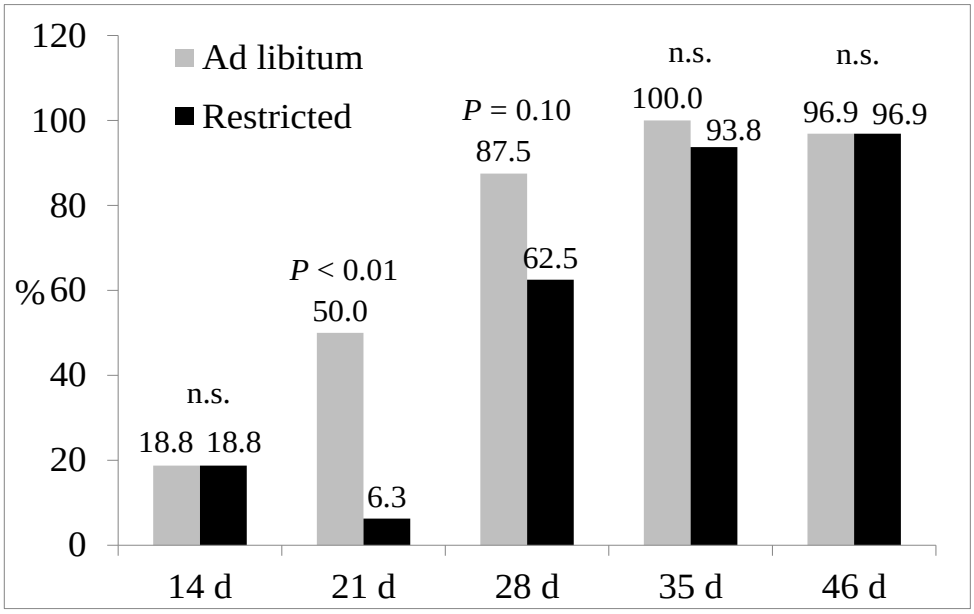

Figure 2.5 Percentage (%) of chickens showing muscle fibre degeneration at histological examination of *P. major* between those fed *ad libitum* or submitted to feed restriction from 13 d to 21 d of age and at different ages (14, 21, 28, 35, and 46 d) (*P*-value of Chi square test within age).

The MFD score was not significantly affected by the genotype, the gender or the feeding system, but it significantly increased with the age of the chick ($P < 0.001$) (Table 2.5). The MFD score also differed between chickens submitted to the two feeding systems depending on the age of observation at a level approaching statistical significance (probability of the interaction feeding system × age; $P = 0.10$): the score was higher in chickens fed *ad libitum* compared to those restricted at 21 d (0.69 *vs.* 0.06) and 28 d of age (1.50 *vs.* 1.13) (data not reported in tables). In contrast, the number of apoptotic nuclei increased only with the chicken age (from 0.09 at 14 d to 8.45 at 46 d of age; $P < 0.001$) (Table 2.5).

Table 2.5 Score for muscle fiber degeneration and number of apoptic nuclei (LS means±SE) in *P. major* of broiler chickens differing for genotype, gender, feeding system and age.

	Breast yield (B)		Gender (G)		Feeding system (F)		Age (A)					*P*-value				
Item	Standard	High	Females	Males	*ad libitum*	Restricted	14 d	21 d	28 d	35 d	46 d	B	G	F	A	F x A
Score[a]	1.16±0.08	1.19±0.08	1.15±0.08	1.21±0.08	1.24±0.08	1.12±0.08	0.19±0.14	0.38±0.14	1.31±0.14	2.09±0.14	1.92±0.10	0.79	0.64	0.32	<0.001	0.10
Apoptic nuclei, n	2.94±0.49	3.39±0.49	2.84±0.49	3.49±0.49	3.21±0.49	3.12±0.49	0.09±0.82	0.44±0.82	3.03±0.82	3.81±0.82	8.45±0.58	0.53	0.36	0.89	<0.001	0.97

[a]Score for muscle fiber degeneration: 0 (normal), no necrotic fibres, no infiltration of connective tissues, normal or central nuclei; 1 (mild) central nuclei, some fibres with hyaline cytoplasm, scarce necrotic fibres, absence of connective tissue infiltration; 2 (moderate), diffuse necrotic fibres, thickening of interstitial connective tissue, presence of inflammatory cells, and appearance of adipose tissue aggregates; 3 (severe), great amount of interstitial connective tissue and inflammatory cells, of necrotic fibres and lobules of adipose tissue.

The above-described evolution of MFD score with age corresponded to the following description at histological analyses: at 14 d of age, most sections of *P. major* exhibited an organized skeletal muscle consisting of single muscle fibres covered by fibrous connective tissue, the endomysium, which insulated each fibre (Figure 2.6A); inside each muscle fibre, numerous longitudinally arrayed myofibrils were visible; nuclei were located peripherally just beneath the sarcolemma which was covered by the endomysium. At 21 d, the histomorphology of muscle tissues showed an increasing range of myodegenerative lesions (Figure 2.6B-D; Figure 2.7A-D) from 1 (mild) to 3 (severe). Most of the muscle parenchyma exhibited a normal structure, although numerous fibres appeared hyper-eosinophilic, with loss of cross striations and internalization of nuclei (Figure 2.6B). Some hyper-eosinophilic fibres exhibited a vacuolar degeneration and rare fragmented fibres undergoing to a phagocytic process and appeared surrounded by inflammatory cells. At 28 d, the muscle parenchyma showed an increasing percentage of degenerating muscle fibres when compared to the previous stage (Figure 2.6C). Moreover, the *interstitium* among muscle fibres appeared infiltrated by inflammatory cells, such as lymphocytes and macrophages. At 35 d of age, muscle fibres appeared surrounded by an abundant collagen-rich connective tissue (Figure 2.6D).

At 46 d, most of the fibres lost the typical cross striations and exhibited a massive necrotic process (Figure 2.7A, 2.7B) and degenerating fibres appeared surrounded by inflammatory cells (Figure 2.7B, 2.7C). At this stage, fibres were scattered in an abundant collagen-rich connective tissue and exhibited a high variability in size (degenerating and regenerating fibres) (Figure 2.7B, 2.7C, 2.7D).

The connective tissue was rich in collagen fibres (Figure 2.8A, 2.8B) and numerous adipose cells (lipidosis) were detectable (Figure 2.8C, 2.8D, 2.8E). Immunological analyses revealed the number of nuclei positive to the anti-cleaved lamin A antibody increased from 14 d to 46 d of age (Figure 2.9A-E). At 46 d, necrotic fibres showed a higher percentage of apoptotic nuclei (Figure 2.9D, 2.9E) and regenerating fibres appeared positive to the anti-PCNA antibody (Figure 2.9F, 2.9G).

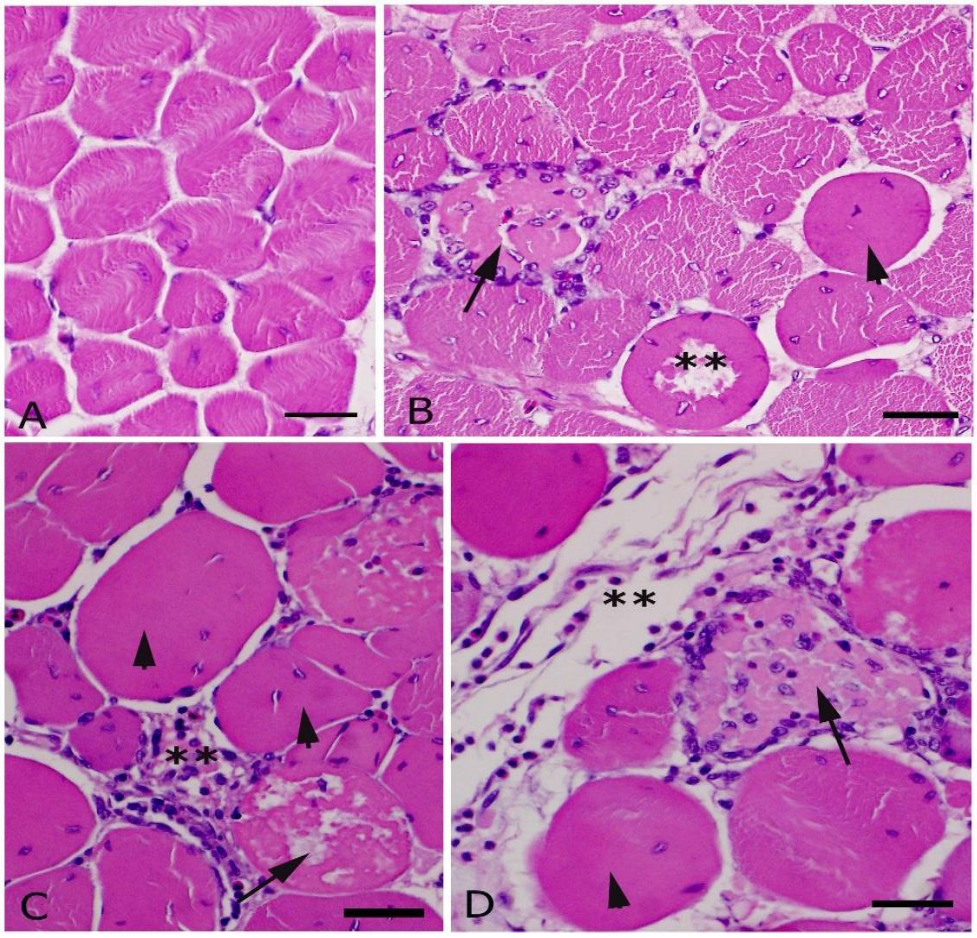

Figure 2.6 Histological evaluation of muscle fibres in chickens at 14 (A), 21 (B), 28 (C) and 35 (D) d of age. All panels are stained with haematoxylin and eosin. A) At 14 d, a normal histological aspect of muscle fibres is visible. In transverse section, the majority of muscle fibres exhibit nuclei arranged peripherally and consist of multi-nucleate cells with a striated pattern. Rare fibres show an internalization of nuclei. B) At 21 d, although most of the muscle parenchyma exhibits a normal structure, numerous muscle fibres appear hyper-eosinophilic (*arrowhead*), whereas others show a vacuolar degeneration (*asterisks*). Rare fibres undergo to phagocytosis, appear fragmented and infiltrated by histiocytes, macrophages and heterophils (*arrow*). C) At 28 d, hyper-eosinophilic fibres are abundant (*arrowheads*) and the *interstitium* is infiltrated by lymphocytes and macrophages (*asterisk*). D) At 35 d, degenerating fibres (*arrow*) are often surrounded by inflammatory cell infiltration and abundant collagen-rich connective tissue (*asterisks*). Arrowhead indicates an hyper-eosinophilic fibre. Scale bars: A, 20 μm; B, C, and D, 10 μm.

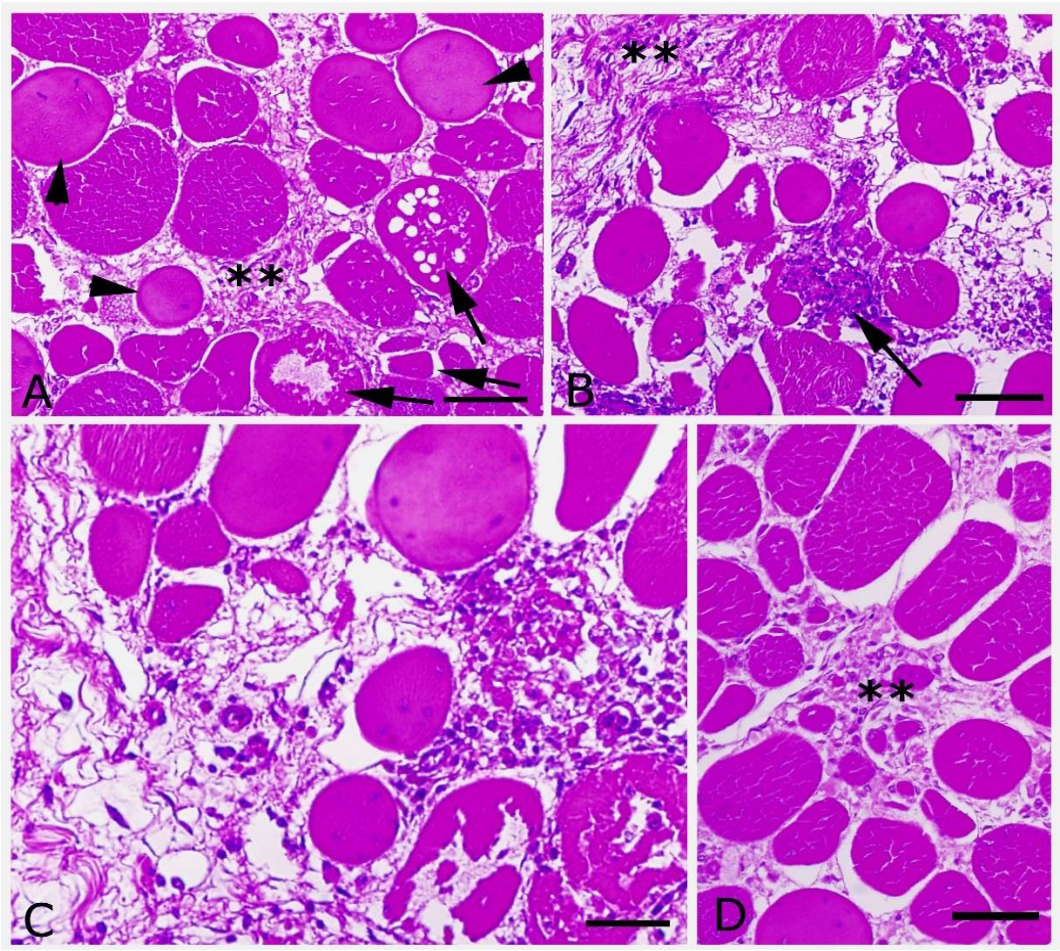

Figure 2.7 Histological evaluation of muscle fibres in chicken aged 46 days. All panels are stained with haematoxylin and eosin. A) A gradual increase in degenerating fibres (*arrows*) scattered among hyper-eosinophilic fibres (*arrowheads*) is detectable. At this stage, the presence of collagen-rich connective tissue (*asterisks*) is higher than in previous stages. B) The number of muscle fibres, which show a variability in diameter size, is reduced when compared to that of previous stages. Fibres are replaced by connective tissue (*asterisks*). Arrow indicates a degenerating fiber infiltrated by inflammatory cells. C) An abundant connective tissue surrounds muscle fibres which exhibit a variability in diameter size, reflecting a regeneration process as evidenced in panel D (*asterisks*). Scale bars: A, B, D, 20 μm; C, 40 μm.

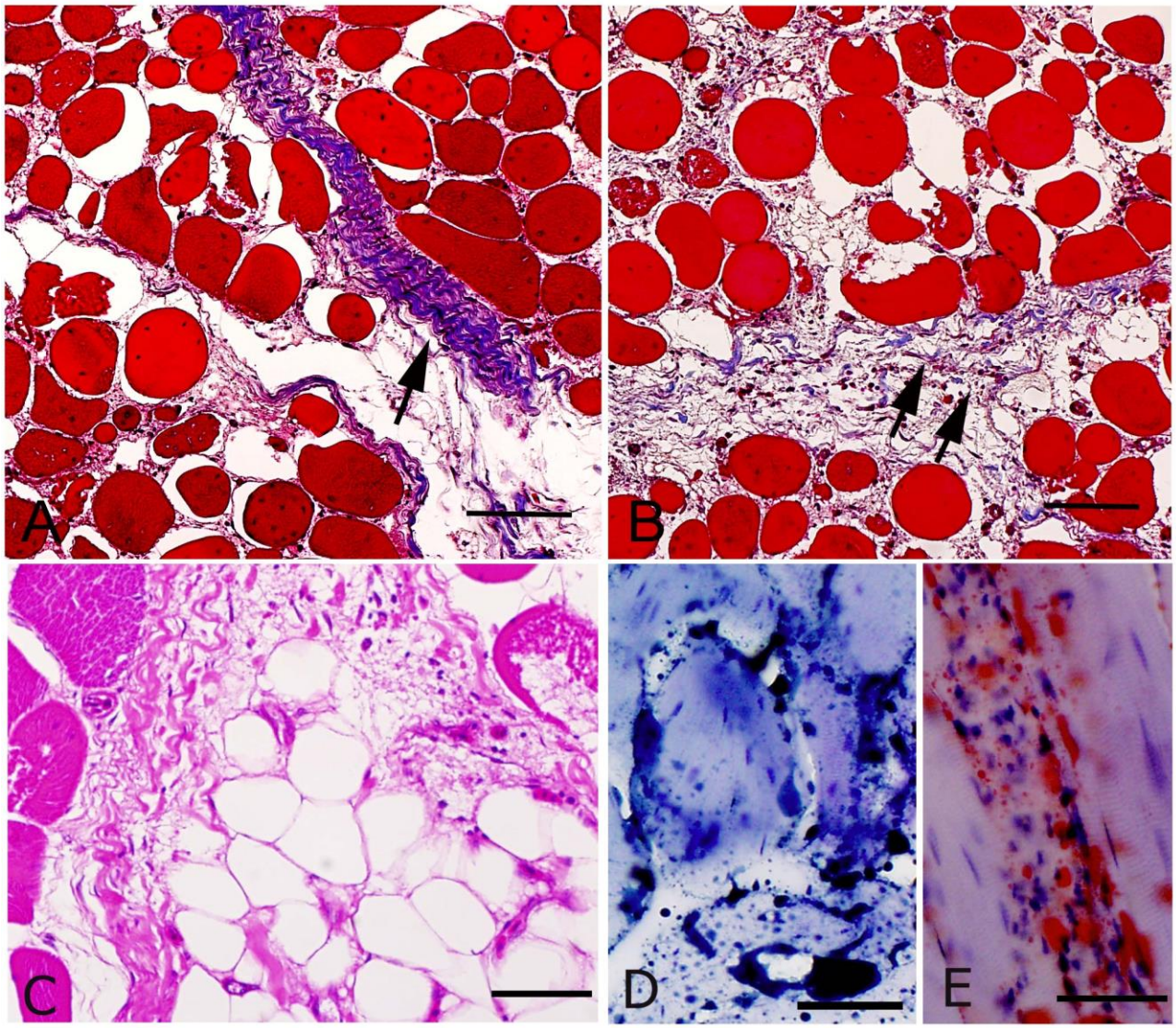

Figure 2.8 Histological evaluation of muscle fibres in chickens at 46 d of age. Panels A and B are stained with Masson's trichrome staining; panel C is stained with haematoxylin and eosin; panel D is stained with Nile blue; panel E is stained with Oil red. A, B) The numerous collagen fibres (*arrows*) distributed in the interstitium among muscle fibres are stained in blue by Masson's trichrome staining. C) Degenerated muscle fibres are also replaced by abundant adipose tissue. D, E) Adipose cells appear stained in blue and red by Nile blue and Oil red staining, respectively. Scale bars: A, B, 40 μm; C, D, and E, 20 μm.

Both genotypes exhibited a similar pattern at immunohistochemistry. The number of microvessels was not affected (P>0.10) by genotype (14.5±0.75 and 15.7±0.72 in standard and high breast yield chickens), gender (15.9±0.71 and 14.3±0.76 in females and males) or feeding system (15.5±0.75 and 14.7±0.72 in chickens fed *ad libitum* and in those restricted from 13 to 21 d) (data not reported in tables). In contrast, the number of microvessels was significantly higher in chickens at 14 d than at 46 d of age (20.7±0.75 *vs.* 9.46±0.72; P < 0.001).

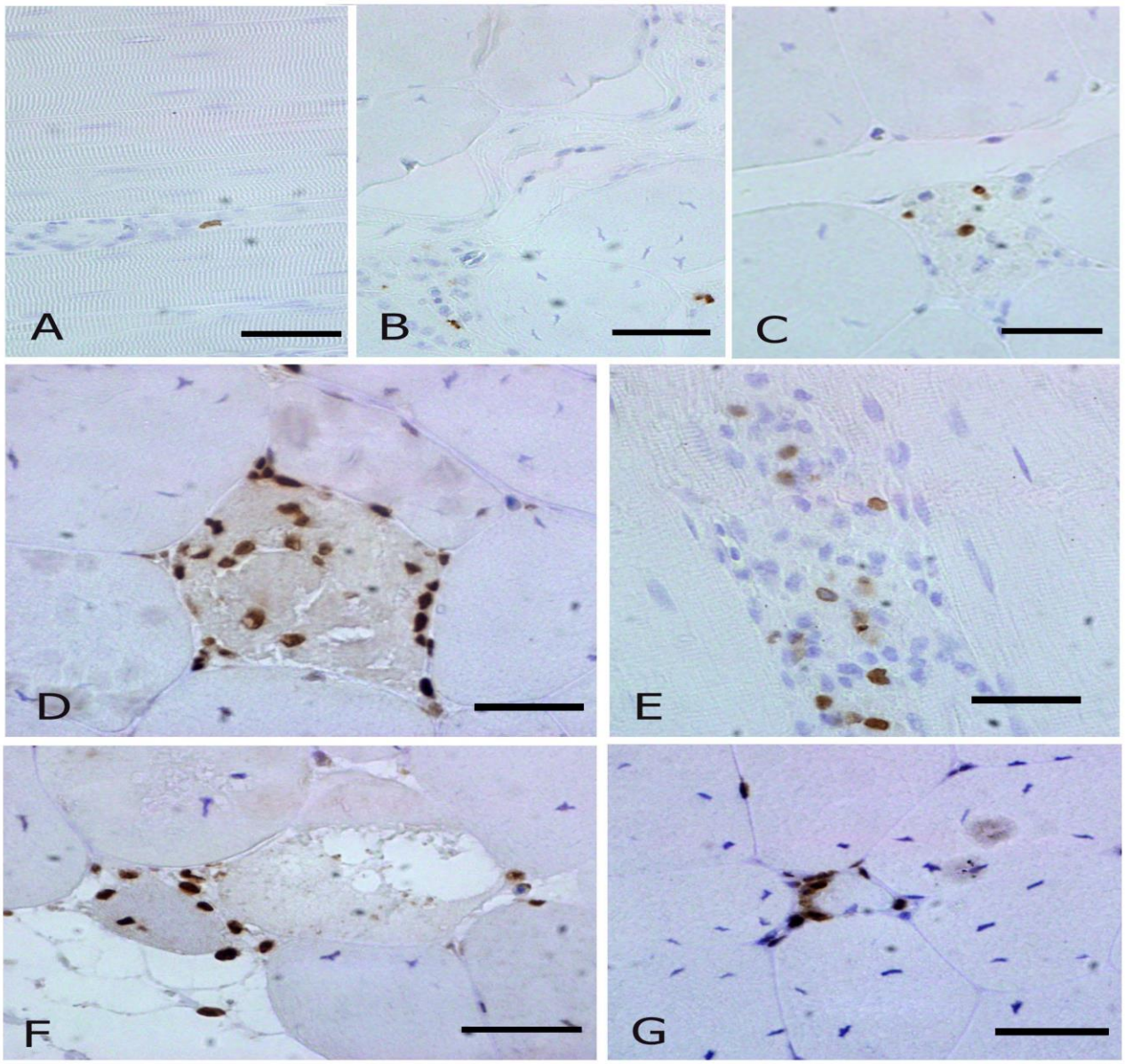

Figure 2.9 Immunohistochemical localization of anti-cleaved lamin A and PCNA in muscle fibres of chicken sampled at 21 (A), 28 (B), 35 (C) and 46 (D) d of age. Panels A-E are immunostained with the anti-cleaved lamin A antibody, whereas panels F and G are stained with the anti-PCNA antibody. A-D) The number of nuclei positive for anti-cleaved lamin A increased during chicken growth, reflecting the increase in the number of degenerating fibres. At 46 d, degenerating fibres (D, E) show abundant nuclei immunopositive for anti-cleaved lamin A, whereas regenerating muscle fibres (F, G) are immunostained with the anti-PCNA antibody. Both genotypes exhibited a similar pattern at immunohistochemistry. Scale bars: A, B, and C, 20 μm; D, E, F, and G, 10 μm.

DISCUSSION

Both genotypes tested in our trial were commercial fast-growing strains, which fully expressed their performance by reaching final live weights (on average 3,168 g at 46 d of age, corresponding to 70.1 g/d daily growth) and feed conversion rates (1.59) during the trial consistent with their standard. Indeed, performance resulted higher than that achievable under field commercial conditions: Lorenzi *et al.* (2014) reported that females and males belonging to standard and high-breast yield genotypes reached 2,676 g and 2,684 g live weight at 47.7 and 48.0 d of age, accounting for a daily growth rate of 56.1 g/d and 56.4 g/d, respectively for the two genders (average values of the two hybrids).

Under our conditions, the standard hybrid always showed a lower feed intake and was somewhat advantaged, especially during the second period of growth in which it maintained a higher growth rate, compared to the high-breast-yield genotype. Also Petracci *et al.* (2013a) found that chickens of the standard genotype reached the slaughter weight (4.2 kg) two days before the high-breast-yield chickens (53 d *vs.* 55 d of age).

The heavier carcasses of the standard genotype showed a higher development of thighs (in percentage of carcasses) compared to the other genotype. The unexpectedly similar breast rate (expressed as percentage of the carcass weight) observed in the chickens of the two genotypes may be due to the different slaughter weight, whereas Petracci *et al.* (2013b) found a lower breast rate in standard chickens compared to high-breast-yield chickens when slaughtered at the same live weight (4.2 kg).

Meat quality is expected to largely vary among genotypes with large genetic differences and different growth rates (Berri *et al.*, 2005; Sirri *et al.*, 2011), but even two genotypes belonging to the same commercial brand may show differences. Petracci *et al.* (2013b) found lower meat pH and lower drip losses, besides large differences in the histological traits of breast muscle, in standard chickens compared to high-breast-yield chickens slaughtered later (53 d and 55 d respectively for the two strains) and heavier (4.2 kg) than our birds.

The higher final pH measured in the *P. major* of the chickens of the standard genotype compared to the high-breast-yield genotype explains the lower breast lightness index, due to the negative correlation between the two traits (Debut *et al.*, 2003).

In the present experiment, meat quality did not differ to an appreciable extent according to gender: pH of *P. major* was lower in females than in males, as previously found by other authors (López *et al.*, 2011; Brewer *et al.*, 2012; Schneider *et al.*, 2012), but lightness and red indexes and water holding capacity were similar; the higher yellow index measured in females than in males has been reported also by Schneider *et al.* (2012).

As expected, the feeding regime conditioned (and impaired) growth rate of chickens during the restriction period, as previously found by several authors adopting different feed restriction plans and systems (Urdaneta-Rincon and Leeson, 2002; Zhan *et al.*, 2007; Butzen *et al.*, 2013). During the re-alimentation period, however, previously restricted birds showed a compensatory growth which allowed birds to reduce differences in final live weight and improved feed conversion rate. Likely, the duration of the re-alimentation

period was not sufficient to permit the full recover of live weight, and some residual effects were still evident in terms of reduced dressing percentage, breast and thighs yield in the restricted birds as reported by others (Urdaneta-Rincon and Leeson, 2002; Zhan *et al.*, 2007; Butzen *et al.*, 2013). As reviewed by Sahraei (2012), in fact, the duration, timing, and severity of feed restriction as well as the re-alimentation terms affect the occurrence and degree of the compensatory growth and, therefore, the residual effects on carcass quality.

The feeding regime affected only final pH, lower in the *P. major* of the chickens fed *ad libitum* compared to those submitted to feed restriction. We could hypothesize that chickens fed *ad libitum* stored more glycogen compared to the restricted ones and, therefore, more lactic acid was available in meat after 24 h (Alnahhas *et al.*, 2014). Among other authors, Butzen *et al.* (2013) did not detect any significant effect of early feed restriction on thawing and cooking losses or shear force, like in our study, whereas Ponte *et al.* (2008) did not find significant effects on final meat pH of chickens fed exclusively on a cereal-based diet even when restricted until slaughter.

Controlling the occurrence and the degree of myopathies would be of great interest for the poultry industry in view of preventing and containing negative effects on consumers acceptance (Kuttappan *et al.*, 2012b) and meat technological properties (Mudalal *et al.*, 2014; Mazzoni *et al.*, 2015). Macroscopically, myopathy occurrence at commercial slaughtering has been found to range from 9.8% of white striped breast fillets in broilers slaughtered at 42 d of age and 3.2 kg live weight (Ferreira *et al.*, 2014) to 12.0% in light birds slaughtered from 45 d to 54 d of age (average live weight: 2.75 kg) (Petracci *et al.*, 2013b) to 55.8% in chickens slaughtered later (59 d to 63 d) (Kuttappan *et al.*, 2013a) or 60.3% in heavy male birds (live weight from 3.8 to 4.2 kg) (Lorenzi *et al.*, 2014) up to 86.7% in chickens slaughtered at 46 d of age (Trocino *et al.*, 2015).

The correlation between white striping occurrence and growth rate/final live weight and/or breast yield in current broiler genotypes under commercial intensive conditions has been proposed but not fully demonstrated. In fact, some authors have found that heavier birds with thicker breasts are most likely to show. High growth rates and high breast yield have also been considered responsible for wooden breasts (Sihvo *et al.*, 2014), but the only significant difference in wooden breast occurrence has been reported between heavy males and light females slaughtered at the same age (Trocino *et al.*, 2015). Indeed, according to Bailey *et al.* (2015), environmental and/or management factors may contribute to more than 65% and 90% of the variance in the occurrence of white striping and wooden breast, respectively. In fact, these authors found that heritability was low both for wooden breast (<0.10) and white striping (<0.34) and genetic correlations between breast myopathies and body weight ranged from less than 0.132 to 0.248.

When the same group of birds was controlled during growth, as in the present study, white striping appeared soon at 14 d of age (9.38% of controlled animals at gross examination) and increased at 21 d, 28 d, and 35 d (31.3%, 78.1, and 74.5%, respectively; data not reported in tables), while wooden breasts were observed only in birds at the last slaughtering at 46 d (74.5% and 12.2% of white striped and wooden breasts, respectively;

Trocino *et al.*, 2015). Moreover, at gross examination the two myopathies were often concomitantly present (Trocino *et al.*, 2015; Soglia *et al.*, 2016).

Whether white striping and wooden breast are different expressions of the same myopathy or not is far to be demonstrated as stated by Velleman (2015), but the histological lesions described are very similar. In fact, in the same animals of the present study, muscles affected by white striping or wooden breast at 46 d did not differ at an histological level and a range of microscopic lesions (i.e. internalization of nuclei, loss of cross striation, vacuolar degeneration and necrosis of fibres, lymphocytes and macrophages infiltration, degenerating and regenerating fibres of variable size, lipidosis and fibrosis) was observed (Trocino *et al.*, 2015), as already reported by other authors (Kuttappan *et al.*, 2013b; Sihvo *et al.*, 2014; Velleman and Clark, 2015) in both white striped and wooden breasts. In addition, in two different genotypes affected by wooden breasts, Velleman and Clark (2015) assessed different collagen distribution and arrangement of collagen fibrils, as well. In our study, at the last slaughtering either regenerating and degenerating fibres were observed, as previously reported by Sihvo *et al.* (2014). In fact, Velleman and Clark (2015) found that wooden breasts had increased expression of the myogenic transcriptional regulatory factors linked to satellite cell–mediated repair of muscle fibre damage, even if with some differences between genetic lines.

At 46 d, necrotic fibres showed the highest percentage of apoptotic nuclei and were surrounded by isolated regenerating fibres which appeared positive to the anti-PCNA antibody in both wooden breast and white striping myopathies. A regenerative process has been also described by Sihvo *et al.* (2014) in wooden breast but not in white striping myopathies.

Both myopathies have been associated with an increased muscle hypertrophy of fast growing chickens which brings about reduced capillary density adjacent to the myofiber (Hoving-Bolink *et al.*, 2000; Joiner *et al.*, 2014) thus affecting regeneration, degeneration and necrosis of skeletal muscles (Velleman, 2015). The alteration of vascular support hinders the satellite cell-mediated repair mechanism resulting in fibrosis (Siller, 1985). In addition, muscle multifocal degeneration and necrosis due to myopathies have been associated with altered Na and Ca metabolism since their levels increase, and fast-twitch skeletal muscle Ca-ATPase is overexpressed in wooden breast and white striped P. major (Soglia *et al.*, 2016). The analysis of gene expression and pathway by RNA-sequencing reasonably support the hypothesis that localized hypoxia, oxidative stress, increased intracellular calcium, and the presence of muscle fibre-type switching may be responsible for wooden breast (Mutryn *et al.*, 2015).

According to our results, the above mentioned damaging mechanisms seem to occur early. In fact, at histological examination, we found that muscle fibre degeneration appeared soon after 14 d of age at an appreciable rate (18.8%) and affected half (50.0%) and nearly all (87.5%) the chickens fed ad libitum within 21 d and 28 d, respectively; at 35 d and 46 d, all breasts examined histologically showed muscle fibre degeneration (100% and 96.9%, respectively). Moreover, the severity of lesions increased as the animal age increased and went together with a strong reduction of the number of small vessels (the most abundant ones).

Differently, Velleman *et al.* (2010) did not observe any muscle fibre degeneration or necrosis in the P. major of chickens fed ad libitum and controlled each three days from hatch to 42 d of age. Indeed, P. major muscles selected at slaughter from heavy male broilers among the normal ones (i.e. without white striping and/or wooden breast at gross examination) showed myofibres with a normal profile, endo- and perimysial connective tissues without relevant alteration and few abnormal fibres (Soglia *et al.*, 2016).

In our study, feed restriction was used to control bird growth and thus muscle fibre accretion from 13 to 21 d of age. Accordingly, during the same period, feed restriction successfully reduced the occurrence of muscle fibre degeneration compared to ad libitum feeding as measured histologically at 21 d of age. During the subsequent re-feeding period, however, restricted birds showed a compensatory growth which induced also an important damage at fibre level as detected at 28 d of age. In addition, at gross examination of muscles at the last slaughtering (at 46 d of age), the occurrence of white striped breasts increased by 10 percentage units in the restricted birds compared to those fed ad libitum (Trocino *et al.*, 2015). Further insight would be necessary to assess whether a gradual re-alimentation program after feed restriction would maintain the benefits of feed restriction on myopathies occurrence. In fact, white striping occurrence in broilers slaughtered at 54 d of age was reduced only when growth rate was controlled during the whole rearing period by the administration of low energy diets (Kuttappan *et al.*, 2012). On the other hand, a too early feed restriction (first two weeks post hatching) had negative effects on P. major structure, in terms of poor organization, increased necrosis, and fat deposition, which were still fully evident at 42 d of age (Velleman *et al.*, 2010). Finally, other strategies based on the dietary administration of antioxidants could play a role in reducing the progressive inflammation and oxidative stress and, thus, muscle fibres degeneration. However, previous results on the dietary supplementation of vitamin E proved a positive effect on the reduction of the average number of degenerated fibres per field in the P. major only at early observations (28 d) which disappeared by 42 d and 49 d of age (Guetchom *et al.*, 2012).

In conclusion, growth performance, carcass, and meat quality may significantly change with genotype, but the extent of differences is quite limited within the tested modern hybrids and not really relevant from an economic point of view. On the other hand, both gender and feed restriction may largely affect growth performance and slaughter traits.

Moreover, muscle fibre degeneration occurred early in broilers after two weeks of growth, increased dramatically within 28 d of age and was detected in almost all animals at histology within the end of the trial (46 d of age) regardless of genotype and gender. Feed restriction from 13 to 21 d of age was effective in controlling and reducing muscle fibre degeneration occurrence only as long as birds were under restriction and did not fully express their growth capability, but no residual positive effect was recorded after the re-alimentation period. This information about the time and factors affecting myopathies occurrence may contribute to arrange a suitable control of production factors in view of reducing the presence and the degree of abnormalities.

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CHAPTER 3: SECOND CONTRIBUTION

Effect of feed restriction strategy on growth, carcass and meat quality and myopathy occurrence in broiler chickens

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GRATTA F., BIROLO M, BALLARIN C., BERTOTTO D., RADAELLI G., PICCIRILLO A., XICCATO G., TROCINO A. 2017. Effect of feeding system and age on muscle fibre degeneration in two broiler chicken genotypes. Proceedings of Egg&Meat2017, 03-05/09/2017, Edinburgh, UK. (oral communication), p.64

ABSTRACT

At total of 900 broiler chickens were reared until slaughter (48 d) to evaluate the effect of genotype and feeding regime (ad libitum-AL *vs.* early restricted-ER, 13 to 23 d of age, and late restricted-LR, 27 to 37 d of age; restriction rate at 80%) on performance, meat quality, myopathy rate and degree, and muscle fiber degeneration (MFD) at different ages (22 d, 36 d, and 48 d). At 48 d of age, AL chickens showed higher live and carcass weights (3482 g *vs.* 339 g; $P < 0.01$) and *P. major* proportion (26.4% *vs.* 25.4%; $P < 0.05$) compared LR birds, whereas ER chickens had intermediate value (3454 g and 25.6%). At gross examination, the feeding system did not affect white striping (WS) or wooden breast (WB) rate or degree at breasts or at hind leg. At 48 d, Ross chickens were heavier than Cobb (3548 g *vs.* 3342 g; $P < 0.001$), but had higher feed conversion rate (1.69 *vs.* 1.62). The former also had higher dressing out percentage (77.9% *vs.* 77.0%; $P < 0.001$), but lower *P. major* proportion (25.6% *vs.* 26.2%; $P < 0.05$), besides higher pH (5.98 *vs.* 5.89; $P < 0.001$), meat cooking losses (26.3% *vs.* 24.5%; $P < 0.01$), and shear force (2.39 kg/g *vs.* 2.12 kg/g; $P < 0.05$), and lower lightness index (42.7 *vs.* 43.8; $P = 0.01$) at *P. major*. The occurrence of severely WS breasts was higher in Ross than in Cobb (25.9% *vs.* 7.41%; $P < 0.001$). The WB occurrence was not affected by the feeding system or the genotype. The MFD score significantly increased with the chicken age (1.25 to 1.88 and 2.42 from 22 d to 37 d and 48 d of age; $P < 0.001$). On average of the three ages, the MFD score was not affected by the feeding system, but differed between genotypes (1.67 *vs.* 2.03 in Cobb *vs.* Ross; $P < 0.001$).

INTRODUCTION

During the last years, research on meat quality in poultry aimed at evaluating the presence and consequences of myopathies, especially white striping and wooden breast, in *P. major* as well as the factors affecting their occurrence. Both myopathies have repercussions on nutritional and technological properties of meat (Mudalal *et al.*, 2015; Mazzoni *et al.*, 2015; Petracci *et al.*, 2017) and have been classified as degenerative myopathies associated with regenerative changes of the breast muscle (Kuttapan *et al.*, 2013a; Sihvo *et al.*, 2014; Velleman and Clark, 2015, Radaelli *et al.*, 2017). At commercial slaughter, white striping is commonly detected even at very high rates, until 90% (Kuttappan *et al.*, 2012; Petracci *et al.*, 2013a; Lorenzi et al, 2014), whereas wooden breast has more variable occurrence rates (Trocino *et al.*, 2015; Tijare *et al.*, 2016; Bowker and Zhuang, 2016).

Several studies have shown a correlation between high growth rate and high breast yield and occurrence or degree of myopathies affecting *Pectoralis major* and other muscles (Kuttapan *et al.*, 2012, 2013b; Lorenzi *et al.*, 2014; Trocino *et al.*, 2015). However, Bailey *et al.* (2015) have calculated low genetic correlations between breast myopathies, body weight, and breast yield, while outlining the high contribution of environmental and/or management factors to the variance in white striping (>65%) and wooden breast (>90%) occurrence in broiler chickens.

In fact, myopathies rates and/or degree may be affected by ontogenetic factors, like genotype, slaughter weight, gender (Lorenzi *et al.*, 2014; Trocino *et al.*, 2015; Kuttapan *et al.*, 2017), and/or those management factors that may modify growth rate. Among these latter ones, firstly feeding strategies based on low energy diets have been investigated (Kuttappan *et al.*, 2012). Then, quantitative (Trocino *et al.*, 2015; Meloche *et al.*, 2018a) or time-limited (Livingston *et al.*, 2018) feed restriction have been used to control growth and reduce myopathies occurrence. Recently, research focused on the effect of decreasing dietary lysine (especially) and amino acids density to control growth and reduce myopathy rates and degrees (Cruz *et al.*, 2017; Bodle *et al.*, 2018; Meloche *et al.*, 2018b).

Some studies also investigated about onset time of myopathies and muscle fiber degeneration (Sihvo *et al.*, 2017; Griffin *et al.*, 2018) and interactions with feeding strategies (Radaelli *et al.*, 2017). Early feed restriction (13 to 21 d of age) successfully reduced muscle fiber degeneration as long as it was used (Radaelli *et al.*, 2015), but tended to increase white striped breasts at commercial slaughter in comparison with *ad libitum* feeding (Trocino *et al.*, 2015). This result was associated to the fast growth rate related to the compensatory growth during the refeeding period. Similarly, Meloche *et al.* (2018b) also highlighted that terms and timing for controlling curve growth by nutritional strategies (digestible lysine density) have a key role in final performance, breast weight final, and myopathy rates and degrees.

Thus, the present study aimed at evaluating if feeding broiler chickens *ad libitum* or at a restricted rate during early (13 to 23 d of age) or late (27 to 37 d of age) growth may affect performance, slaughter and carcass traits, meat quality, and myopathies rates in male chickens belonging to two genetic lines. Moreover, muscle fiber degeneration (MFD) was recorded histologically at different ages to evaluate interactions with timing of growth control by feeding strategies.

MATERIALS AND METHODS

Experimental facilities

The trial was performed at the poultry house of the Experimental Farm "Toniolo" of the University of Padova (Legnaro, Padova, Italy) during the period May to June, after a long period of downtime (<6 months). The poultry house was equipped with a cooling system, forced ventilation, radiant heating and controlled light systems. Thirty-six wire-net pens (125 cm wide x 177 cm large x 120 cm height; 2.2 m^2) were available, each equipped with an automatic circular drinker (diameter: 39 cm) and a circular feeder (diameter: 37 cm) for manual distribution of feed. The pens had a concrete floor bedded by wood shavings litter (height 5 cm, 2.5 kg/m^2).

Twenty-four hours of light were provided during the first two days after chicks arrival; afterward, hours of lights were progressively reduced to reach and maintain a 18L:6D light program from the 12^{th} day onwards.

Animals, experimental groups and in vivo recordings

A total of 900 male chickens, half Cobb 500 and half Ross 308, were used for the specific aims of the present study, delivered by authorized transport means at the experimental facilities of the University on the hatching day. All chicks had been vaccinated against Marek's disease, Infectious Bronchitis, and Newcastle disease at the hatchery. At their arrival, 25 chicks per pen were housed, randomly allocated to 6 experimental groups, *i.e.* 3 feeding plans (*ad libitum vs.* early restricted *vs.* late restricted) x 2 genotypes, and controlled from the day after their arrival until slaughtering at 48 d of age. Chicks were individually weighed the day after their arrival, identified by a leg mark, and controlled for live weight once a week until slaughtering. Pen feed consumption was measured daily during the trial.

At 22 d and 36 d of age and at live weight equal to 1105±147 g and 2495±227 g, respectively, 36 chickens (one chicken per pen) were slaughtered by cervical dislocation to sample muscles for histological analyses. At 48 d of age, among chickens submitted to commercial slaughter, 72 animals (2 birds per pen) (3483±310 g) were selected as representative in terms of average live weight and variability of the corresponding pens and used to sample *P. major*.

Sampling and histological analyses

At each slaughter, *P. major* muscles were immediately sampled for histology. Samples were fixed in 10% buffered neutral formalin at 4°C overnight, washed in phosphate-buffered saline (PBS, 0.1 M, pH 7.4), dehydrated through a graded series of ethanol and embedded in paraffin.

Sections were cut at a thickness of 4 μm using a microtome (cryostat for frozen samples) and stained with hematoxylin and eosin (H&E) to evaluate the general morphology of the tissues.

At microscopy examination (Olympus Vanox photomicroscope, Japan), myopathic lesions, lipidosis and fibrosis were assessed using a score ranging from 0 to 3 (0, normal; 1, mild; 2, moderate; 3, severe) as developed by Radaelli *et al.* (2017). In details, the score (0) was attributed to samples presenting no necrotic fibers, no infiltration of connective tissue, and with normal or central nuclei; the score (1) was used when samples showed central nuclei, some fibers with hyaline cytoplasm, scarce necrotic fibers, absence of connective tissue infiltration; the score (2) was given when samples diffusely presented necrotic fibers, thickening of interstitial connective tissue, presence of inflammatory cells, and appearance of adipose tissue aggregates; finally, the highest score (3) was attributed to samples exhibiting a great amount of interstitial connective tissue and inflammatory cells, as well as of necrotic fibers and lobules of adipose tissue.

Diets and feeding plans

Three commercial diets were administered during the trial, *i.e.* diet P1 (crude protein 20.1%, ether extract 5.05%; crude fiber 1.19%; ashes 5.19%) from 0 to 23 d, diet P2 (crude protein 18.9%, ether extract 4.93%; crude fiber 1.19%, ashes 5.50%) from 24 to 37 d, and diet P3 (crude protein 17.0%, ether extract 5.37%; crude fiber 1.34%, ashes 5.26%) from 38 d until slaughtering. Diets were produced by a commercial feed mill (Martini, Budrio di Longiano, Italy). Twelve pens were fed *ad libitum* during the experimental trial; other 12 pens were restricted in the period from 13 to 23 d of age (early restricted); the remaining 12 pens were restricted from 27 to 37 d of age (late restricted). The restricted birds received the 80% of the quantity consumed by the chickens fed *ad libitum* on the previous day. The restriction program was calculated separately on the two genotypes.

Commercial slaughtering, carcass and meat quality recordings

At 48 d of age, all birds were slaughtered in a commercial slaughterhouse, after about 7 hours of feed withdrawal and about 4 hours of water withdrawal. Birds were individually weighed before crating. All birds of a pen were loaded in a transport cage (62.6 cm wide x 160 cm long x 25.0 cm high; 1 m^2). Loading took about one hour; transport from the experimental facilities to the commercial slaughterhouse about 15 min;

lairage before slaughtering about 3 hours. Birds were slaughtered according to the standard practice of the commercial slaughterhouse. Ready-to-cook carcass were recovered after 2 hours of refrigeration at 2°C and individually weighed to measure slaughter dressing percentage.

A total of 216 carcasses (6 per pen), that had been previously selected on the basis of the slaughter live weight to be representative within a pen, were submitted to gross examination to evaluate the occurrence (presence or absence) and the degree (normal, moderate, severe) of white striping on *Pectoralis major* muscle and thighs (*iliotibialis* muscle) (Kuttappan *et al.*, 2012; Kuttappan *et al.*, 2013a, and the occurrence (presence or absence) of wooden breast (Sihvo *et al.*, 2014). Afterwards, out of the 216 carcasses, 108 (3 per pen) were further selected, as representative of the average live weight and variability of each pen, and transported to the Department laboratories to be stored at 2°C before meat quality analyses. Twenty-four hours after slaughter, carcasses were dissected for the main cuts (breast, wings, thighs, and drumstick); *Pectoralis major* muscles were separated from the breasts to be submitted to meat quality analyses (Petracci and Baéza, 2011). The pH of the *P. major* muscles were measured in triplicate on their ventral side with a pH meter (Basic 20, Crison Instruments Sa, Carpi, Italy) equipped with a specific electrode (cat. 5232, Crison Instruments Sa, Carpi, Italy). The L*a*b* color indexes were measured in triplicate in the ventral side of the same muscles covered by a transparent plastic film, using a Minolta CM-508 C spectrophotometer (Minolta Corp., Ramsey, NJ, USA) (Petracci and Baéza, 2011).

Thereafter, one meat portion (8 cm x 4 cm x 3 cm) was separated from the cranial side of *P. major*, parallel to muscle fibers directions, and stored under vacuum in plastic bags at -18°C until the meat analyses. Thawing and cooking losses were measured in this cut (Petracci and Baéza, 2011). After thawing, the meat portion was put in plastic bags and cooked in a water bath for 45 minutes, until an internal temperature of 80°C was achieved. After a 40-minutes cooling, a further meat portion (4 cm x 2 cm x 1 cm) was separated to assess the maximum shear force with LS5 dynamometer (Lloyd Instruments Ltd, Bognor Regis, UK) using the Allo-Kramer (10 blades) probe (load cell: 500 kg; distance between the blades: 5 mm; thickness: 2 mm; cutting speed: 250 mm/min) (Mudalal *et al.*, 2015).

Statistical analysis

Individual data of live weight and daily growth were analyzed by ANOVA with feeding system, genotype, and their interaction as main factors of variability and with pen as a random effect, by the PROC MIXED of SAS software (SAS Institute Inc., 2009). Cage data for feed intake and feed conversion were analyzed by ANOVA with feeding system, genotype, and their interactions as main factors of variability, and by the PROC GLM (SAS Institute Inc., 2009). The frequency of myopathies at commercial slaughter was analyzed with the CATMOD PROC (SAS Institute Inc., 2009) according to feeding system, genotype, and their interactions. Differences between the means with $P \leq 0.05$ were accepted as close to statistical significance.

The scores for muscle fiber degeneration were analyzed by PROC GLIMMIX (SAS Institute Inc., 2009) with genotype, feeding system, age, and their interactions as fixed effects. The Bonferroni t-test was used for means comparison.

Differences between the means with $0.05 < P \leq 0.10$ were accepted as close to statistical significance.

RESULTS

Growth performance

During the first growth period, the chickens submitted to early feed restriction grew less (daily weight gain: -15%; $P < 0.001$) and, thus, weighed less at 22 d of age (-15%; $P < 0.001$) than the chickens of the other two feeding groups as a consequence of their lower feed intake (-16%; $P < 0.001$) (Table 1). During the second period, the early-restricted chickens grew more that those always fed *ad libitum* (+5%) or submitted to late feed restriction (+9%) ($P < 0.001$) consistently with differences in feed intake (Table 1). On the other hand, during the same period, the late restricted chickens grew less than those always fed *ad libitum* (-4%; $P < 0.001$). Thus, at the end of the trial, at 48 d of age, chickens always fed *ad libitum* showed higher live weight compared to those submitted to late feed restriction (+2%; $P < 0.01$), whereas early restricted chickens had intermediate values (Table 1). The feeding system did not affect mortality rate.

The two genotypes significantly differed since the hatching day (Table 1). In the whole trial, the Ross chickens grew (+6%) and ate (+10%) more and, thus, were heavier (+6%) at 48 d of age than the Cobb ones ($P < 0.001$) (Table 1). However, the Ross chickens had a higher feed conversion rate compared to the Cobb ones (+4%) (Table 1). On the other hand, mortality rate was significantly lower in the former than in the latter ($P < 0.001$) (Table 1).

Table 2.1. Productive performance[1] (LS means) of broiler chickens until slaughtering

Item	Feeding system (F)			Genotype (G)		*P*-value			MSE
	ad libitum	Early restricted	Late restricted	Cobb	Ross	F	G	F×G	
Chickens (n)	238	242	238	340	378				
Live weight (g)									
On day 1	51.5	51.8	51.3	49.2	53.8	0.30	<0.001	0.99	3.95
On day 22	1056^{B}	903^{A}	1059^{B}	987	1025	<0.001	<0.001	0.10	93
On day 48	3482^{b}	3454^{ab}	3399^{a}	3342	3548	<0.01	<0.001	0.65	278
First period (1-22 d)									
Weight gain (g/d)	47.8^{B}	40.5^{A}	48.0^{B}	44.7	46.3	<0.001	<0.001	0.10	4.36
Feed intake (g/d)	61.0^{B}	50.8^{A}	60.2^{B}	56.5	58.2	<0.001	<0.05	0.41	2.33
Feed conversion	1.27	1.25	1.25	1.26	1.25	0.13	0.49	0.73	0.03
Second period (23-48 d)									
Weight gain (g/d)	93.6^{B}	98.3^{C}	90.2^{A}	90.7	97.2	<0.001	<0.001	0.34	9.10
Feed intake (g/d)	177^{B}	182^{C}	171^{A}	168	186	<0.001	<0.001	0.56	3.78
Feed conversion	1.88	1.84	1.88	1.83	1.90	0.07	<0.001	0.09	0.04
Whole trial (1-48 d)									
Weight gain (g/d)	73.0	72.4	71.2	70.1	74.3	0.05	<0.001	0.65	5.90
Feed intake (g/d)	122^{B}	120^{AB}	118^{A}	114	126	<0.01	<0.001	0.30	2.91
Feed conversion	1.66	1.65	1.64	1.62	1.69	0.58	<0.001	0.11	0.04
Mortality[2] (%)	13.8	12.3	13.8	17.9	8.70	0.87	<0.001	<0.05	-

MSE, root mean square error; SEM is equal to MSE/√n.

[1]Individual data: live weight and daily growth rate. Pen data: feed intake and feed conversion.

[2]Interaction Feeding system x Genotype. Mortality: 17.6% and 9.33%, 14.4% and 12.7%, 22.2% and 6.00% in Cobb and Ross chicks fed *ad libitum*, Cobb and Ross chicks submitted to early restriction, and Cobb and Ross chicks submitted to late restriction.

Slaughter results and meat quality

The carcass weights changed consistently with final live weights: chickens always fed *ad libitum* showed higher carcass weights (+3%; $P < 0.01$) and *P. major* proportion on the carcass (+4%; $P < 0.05$) compared to late-restricted birds, whereas early-restricted chickens showed intermediate values (Table 2). Regarding meat quality, lightness index was significantly lower in the *P. major* of chickens always fed *ad libitum* than in those submitted to early or late feed restriction ($P < 0.01$); redness index was higher in the meat of early restricted chickens compared to late restricted ones ($P < 0.01$) (Table 3).

Ross chickens had heavier carcasses and showed higher dressing out percentage ($P < 0.001$), but lower breast yield and *P. major* proportion ($P < 0.05$) in comparison with Cobb chickens (Table 2). The former birds also showed higher pH ($P < 0.001$), meat cooking losses ($P < 0.01$), and shear force ($P = 0.01$), and lower lightness index ($P = 0.01$) at *P. major* in comparison with the latter (Table 3).

Table 2.2. Slaughter results and carcass traits (LS means±SEM) in chickens slaughtered at 48 d

Item	Feeding system (F)			Genotype (G)		*P*-value			MSE
	ad libitum	Early restricted	Late restricted	Cobb	Ross	F	G	F×G	
Chickens (n)	238	242	238	340	378				
Cold carcasses (g)	2699	2669	2624	2570	2758	<0.01	<0.001	0.97	230
Dressing out percentage (%)	77.6	76.4	77.3	77.0	77.9	0.42	<0.001	<0.01	2.13
Dissected carcasses (n)	36	36	36	54	54				
Breast yield (% CC)	39.9	39.6	39.3	40.0	39.2	0.26	<0.05	0.97	1.64
P. major (% CC)	26.4^{b}	25.6^{ab}	25.4^{a}	26.2	25.6	<0.05	<0.05	0.95	0.78
Wings (% CC)	10.0	10.1	9.99	10.0	10.0	0.53	0.96	0.52	0.57
Thighs (%CC)	15.2	14.8	15.2	15.0	15.1	0.08	0.80	0.46	0.99
Drumsticks (% CC)	13.0	13.3	13.4	13.3	13.2	0.08	0.48	0.29	0.71
Hind legs (% CC)	28.2	28.0	28.6	28.3	28.2	0.25	0.86	0.77	1.46

MSE, root mean square error; SEM is equal to MSE/√n.

Table 2.3. Rheological traits (LS means±SEM) of *Pectoralis major* muscle in chickens slaughtered at 48 d of age

Item	Feeding system (F)			Genotype (G)		*P*-value			MSE
	ad libitum	Early restricted	Late restricted	Cobb	Ross	F	G	F×G	
Carcasses (n)	36	36	36	54	54				
pH	5.97	5.91	5.93	5.89	5.98	0.22	<0.001	0.55	0.13
L*	42.2^{A}	43.7^{B}	43.9^{B}	43.8	42.7	<0.01	0.01	0.53	2.20
a*	0.64^{AB}	0.79^{B}	0.32^{A}	0.57	0.60	<0.01	0.80	0.76	0.58
b*	16.2	16.8	16.0	16.6	16.1	0.09	0.10	0.84	1.71
Thawing losses (%)	8.61	9.08	8.40	8.82	8.57	0.51	0.60	0.23	2.52
Cooking losses (%)	25.7	25.8	24.6	24.5	26.3	0.17	<0.01	0.78	3.01
Shear force (kg/g)	2.39	2.26	2.11	2.12	2.39	0.18	<0.05	0.06	0.62

MSE, root mean square error; SEM is equal to MSE/√n.

Myopathies rate at commercial slaughter

At gross examination, white striping rate at breasts (Figure 2.1, a) and hind leg (Figure 2.2, a) tended to be the lowest in chickens submitted to early feed restriction ($P \leq 0.10$). Regarding genotypes, the occurrence of severely white-striped breasts was significantly higher in Ross than in Cobb chickens (25.9% *vs.* 7.41%; $P<0.001$) (Figure 1, a), whereas white striping rates at hind leg were similar (Figure 2.2, a). Finally, the occurrence of wooden breast was not significantly affected by the feeding system or the genotype (Figure 2.3).

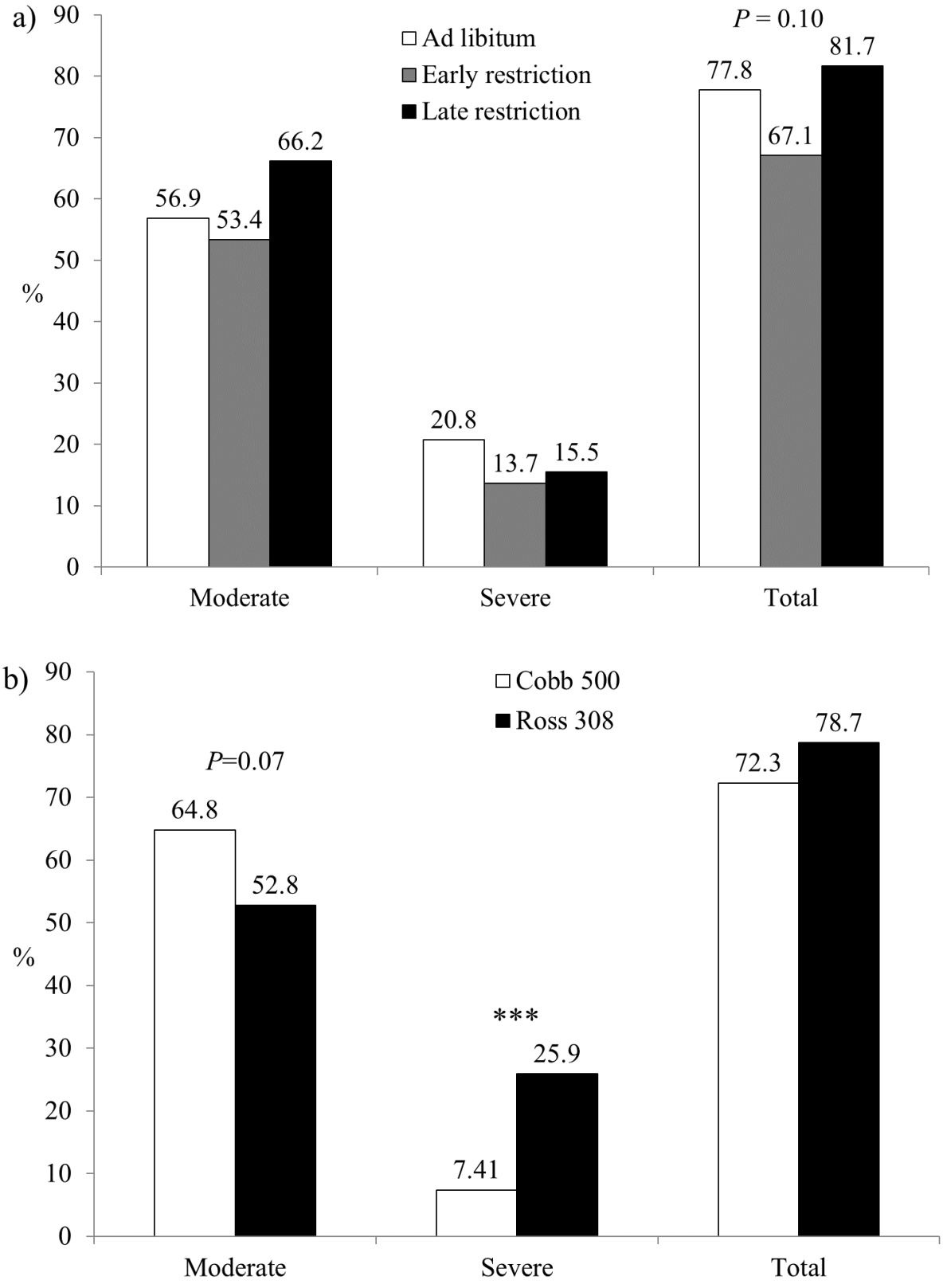

Figure 2.1. Percentage of chickens showing white striping (moderate, severe, total) of *Pectoralis major* at gross observation performed at commercial slaughter (48 d of age): effect of feeding regime (*ad libitum vs.* early restricted *vs.* lately restricted) (a) and genotype (Cobb *vs.* Ross) (b).

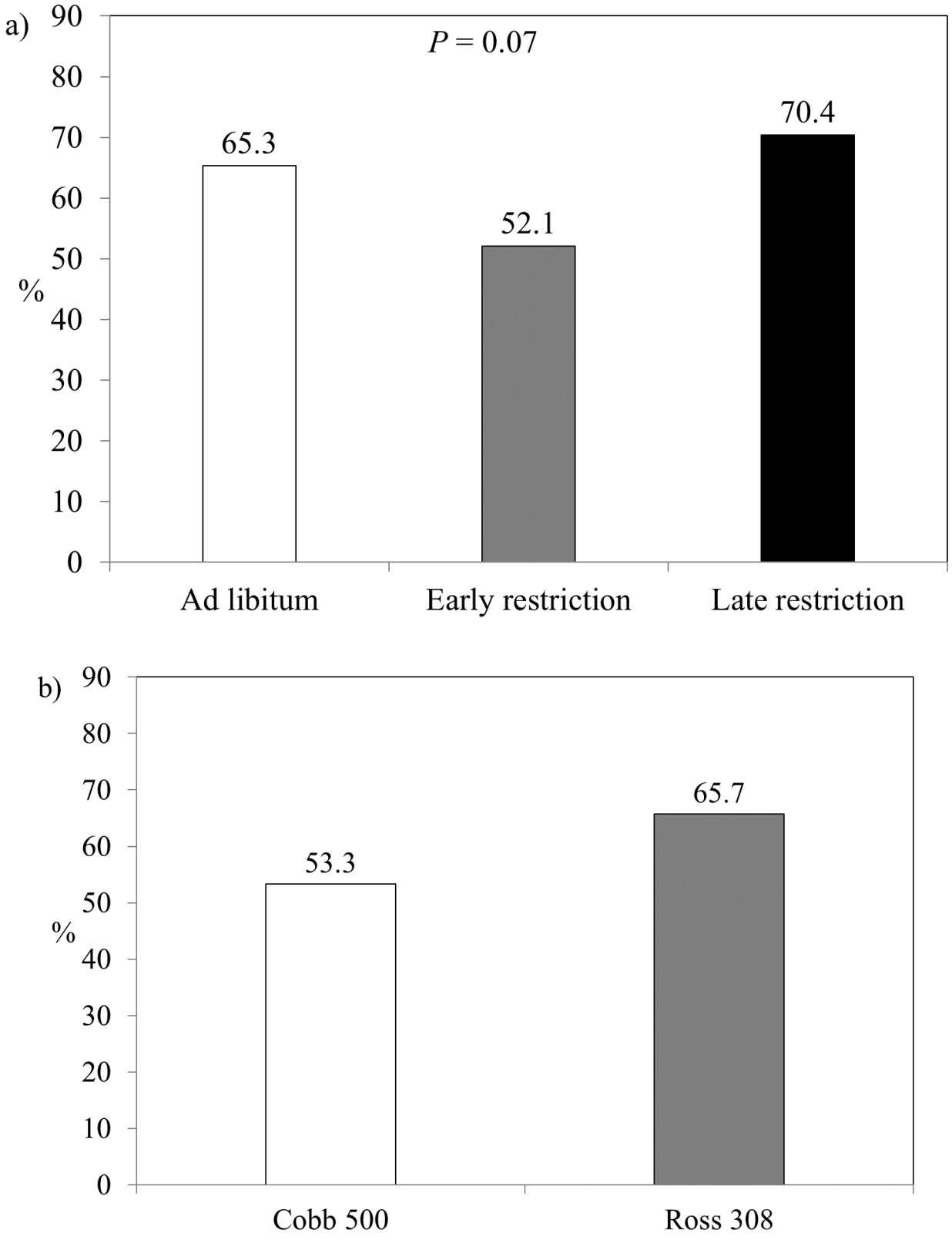

Figure 2.2 Percentage of chickens showing white striping of thighs at gross observation performed at commercial slaughter (48 d of age): effect of feeding regime (*ad libitum vs.* early restricted *vs.* latelyrestricted) (a) and genotype (Cobb *vs.* Ross) (b).

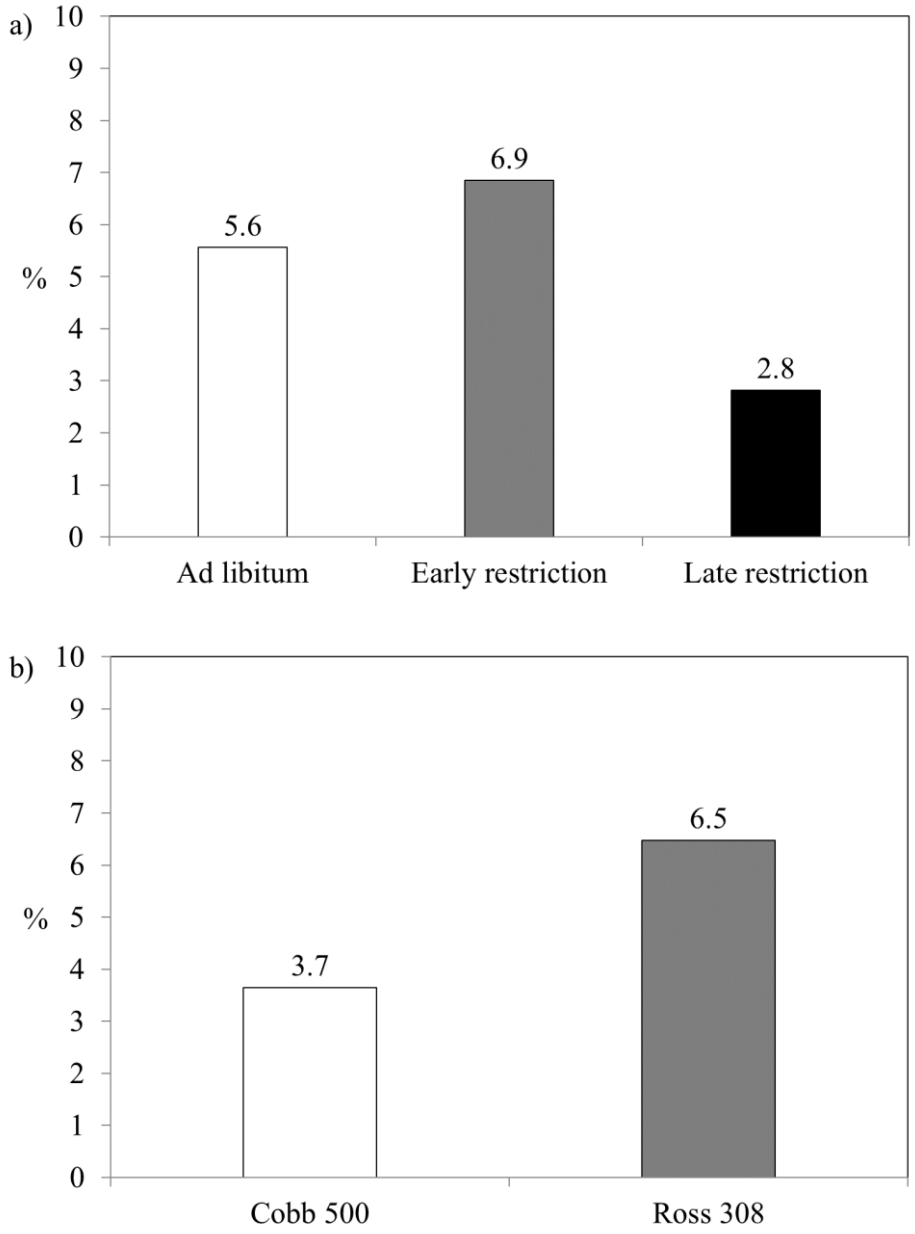

Figure 2.3 Percentage of chickens showing wooden breast of *Pectoralis major* at gross observation performed at commercial slaughter (48 d of age): effect of feeding regime (*ad libitum vs.* early restricted *vs.* lately restricted) (a) and genotype (Cobb *vs.* Ross) (b).

Muscle fiber degeneration

At histological analyses, sections of *P. major* scored as normal (score: 0) exhibited an organized skeletal muscle consisting of single muscle fibers covered by fibrous connective tissue, the endomysium, which insulated each fiber (Figure 2.4A); inside each muscle fiber, numerous longitudinally arrayed myofibrils were visible; nuclei were located peripherally, just beneath the sarcolemma which was covered by the endomysium, and tended to move towards fiber center only occasionally. Adipose tissue between muscle

fibers was absent or very low, whereas inflammatory cells (lymphocytes and macrophages) or necrotic fibers were absent at all.

In sections exhibiting mild MFD (score: 1), most of the muscle parenchyma exhibited a normal structure, although numerous fibers appeared hyper-eosinophilic, with loss of cross striations and in most cases internalization of nuclei (Figure 2.4B). Adjacent muscle fibers were separated by a higher quantity of connective tissue than the control, whereas inflammatory cells and adipose tissue were yet absent. In sections moderately degenerated (score: 2), muscle fiber structure was altered; hyper-eosinophilic fibers were detected which exhibited a vacuolar degeneration and rare fragmented fibers undergoing to a phagocytic process and appeared surrounded by inflammatory cells. The muscle parenchyma showed an increasing percentage of degenerating muscle fibers when compared to the previous stage as well as a higher collagen content (Figure 2.4C). Moreover, the *interstitium* among muscle fibers appeared infiltrated by inflammatory cells, such as lymphocytes and macrophages. Finally, in sections with severe MFD (score: 3), most of the fibers lost the typical cross striations and exhibited a massive necrotic process (Figure 2.4D-E). Fibers were scattered in an abundant collagen-rich connective tissue and exhibited a high variability in size (degenerating and regenerating fibers). The connective tissue was rich in collagen fibers and numerous inflammatory and adipose cells were detectable.

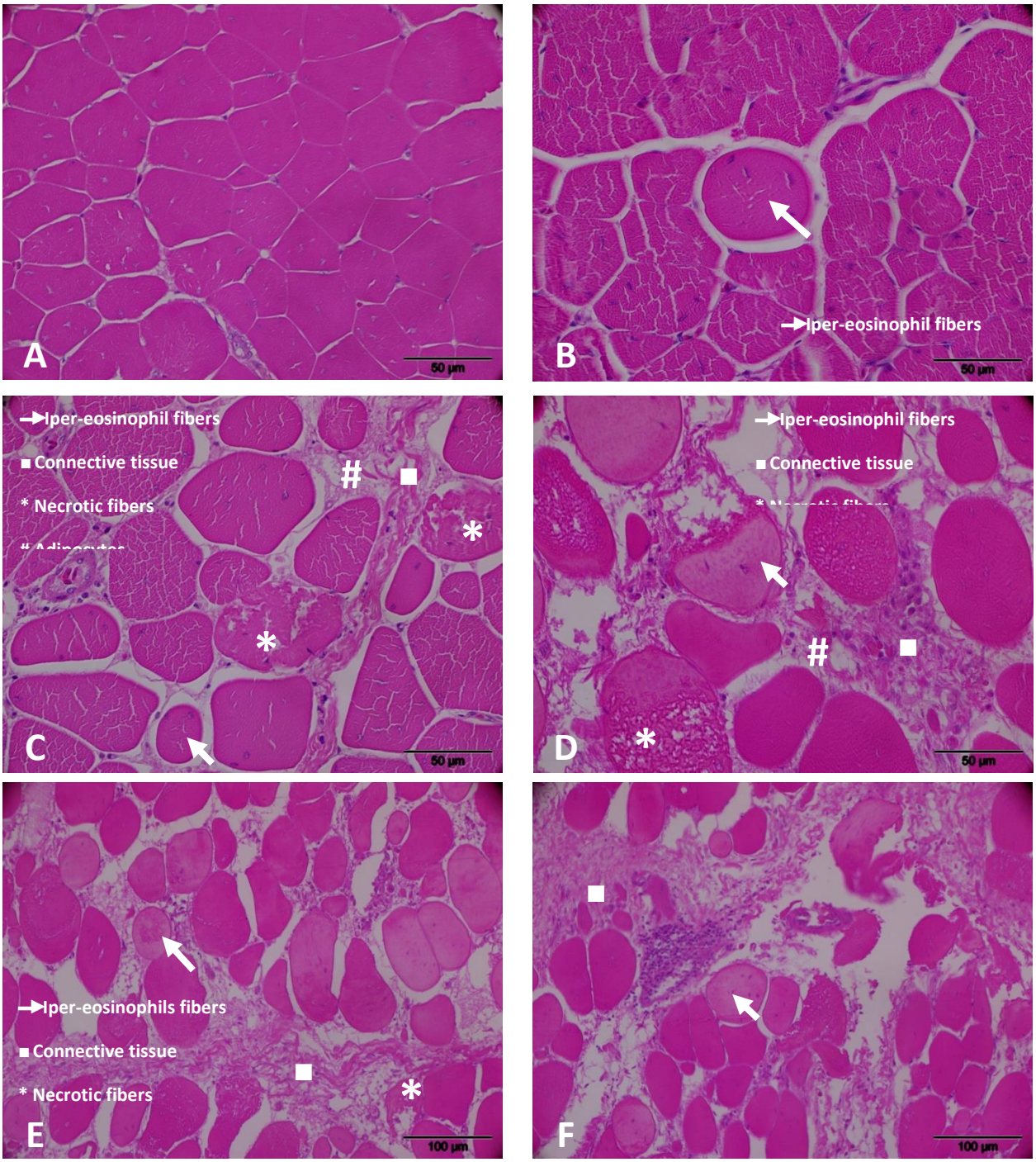

Figure 2.4. Sections of *P. major* scored as normal (A), mild (B), moderately (C) and severely degenerated (D-E). All panels are stained with haematoxylin and eosin. Sections A-B were sampled at 22 d of age; sections C-E were sampled at 48 d of age.

The MFD score significantly increased with the age of the chicks from 1.25 to 1.88 and 2.42 from 22 d to 37 d and to 48 d of age, respectively ($P<0.001$) (data not reported in tables). On average of the three ages, the MFD score was not affected by the feeding system (1.93, 1.81, and 1.81 in *ad libitum*, early-restricted, and late-restricted chickens; $P > 0.10$) but differed between the two genotypes (1.67 *vs.* 2.03 in Cobb *vs.* Ross;

$P < 0.001$) (data not reported). Nevertheless, probability of the interactions of feeding system and genotype with age approached statistical significance ($P = 0.07$ and 0.06, respectively). Regarding the first interaction, the lowest MFD score (1.06) was recorded at 23 d of age in early-restricted birds; the highest MFD scores were measured in chickens always fed *ad libitum* at 36 d of age (2.14) and in all groups at 48 d; moreover, at 36 d of age, MFD was significantly lower in early- and late-restricted birds compared to chickens fed ad *libitum* (1.92 and 1.58 *vs.* 2.14) (probability of Bonferroni comparisons, $P < 0.05$) (Figure 5, a). Regarding the second interaction, at 36 d of age, MFD score differed in the two genotypes (1.54 in Cobb *vs.* 2.22 in Ross chickens) (probability of Bonferroni comparisons, $P < 0.05$) (Figura 5, b).

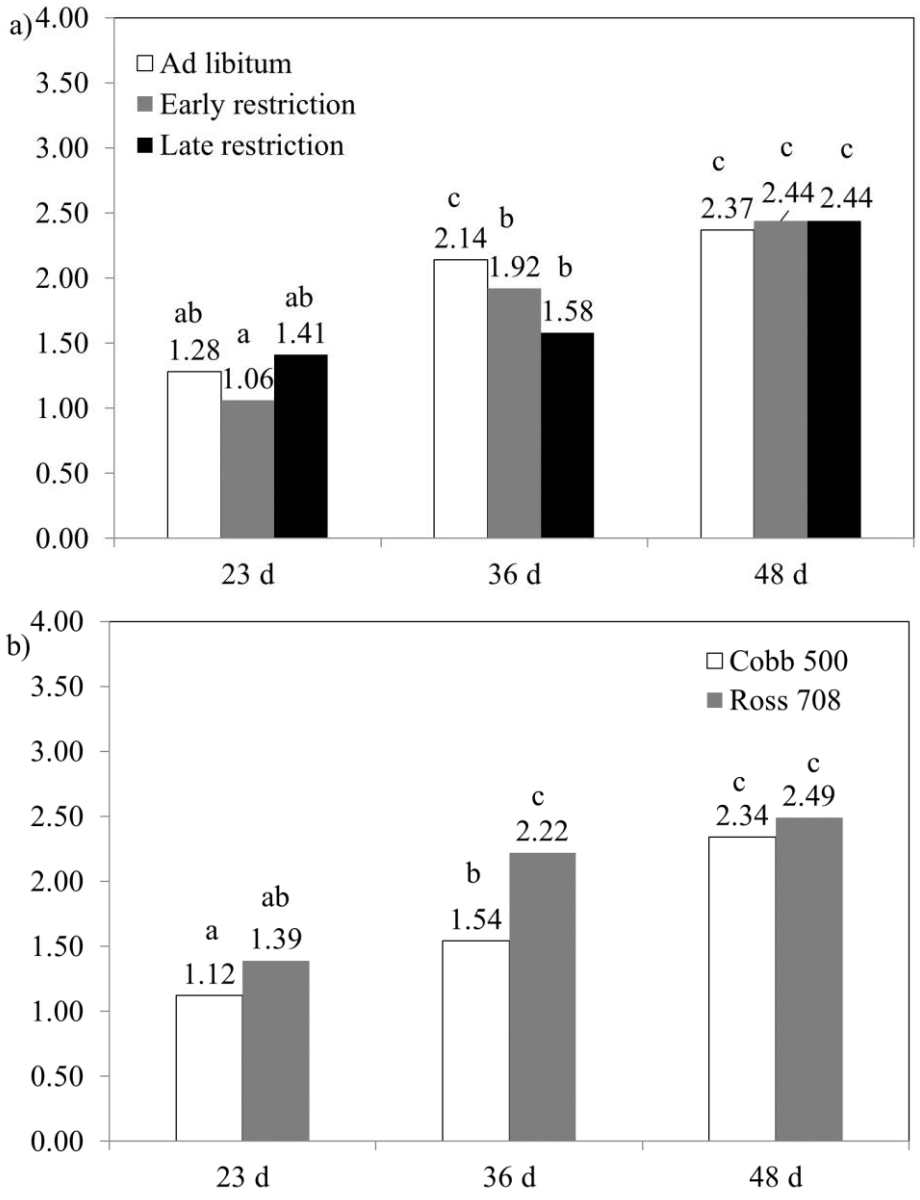

Figure 2.5. Muscle fiber degeneration score measured at *Pectoralis major* at 23 d, 26 d and 48 d of age: effect of feeding regime (*ad libitum vs.* early restricted *vs.* lately restricted) (a) and genotype (Cobb *vs.* Ross) (b). Bars with different letters statistically differ (Bonferroni multiple comparisons $P < 0.05$).

DISCUSSION

In the case of broilers, feed restriction is used to control bird growth curve and occurrence of some metabolic disorders and diseases linked to the high growth rate of selected genotypes (De Jong *et al.*, 2012; Sahraei, 2012; Butzen *et al.*, 2013). Nevertheless, restriction programs should guarantee productive performance and carcass quality as for *ad libitum* feeding. Accordingly, restriction rates, duration, and timing are designed to properly exploit the animal compensatory growth in the refeeding period, pursuing the improvement of feed conversion without detrimental effects on growth performance, carcass traits, and meat quality.

Obviously, feed restriction affects growth rate as long as used: continuous time-limited feed restriction from 7 d to 42 d (Livingston *et al.*, 2018) or quantitative feed restriction (Meloche *et al.*, 2018a) decreased final body weight of chickens, whereas improved global feed conversion ratio. In the present trial, feed restriction impaired the growth rate of early-restricted birds during the first period (0-22 d; restriction from 13 to 22 d) and that of late-restricted birds during the second period (23-47 d; restriction from 23 to 37 d of age). In the case of early-restricted chickens, the refeeding period was sufficiently long for fully express their compensatory growth, so that their final body weight and overall performance (1-48 d) did not differ from *ad libitum* chickens. These results are consistent with previous researches adopting different feed restriction plans and systems (Urdaneta-Rincon and Leeson, 2002; Zhan *et al.*, 2007; Butzen *et al.*, 2013). However, this was not the case of late-restricted chickens of the present trial, which showed lower final body weight and overall performance (1-48 d) than *ad libitum* and early-restricted chickens. Indeed, in a previous trial of ours, even early-restricted males and females chickens (13-21 d of age) slaughtered at 46 d of age approached but did not completely recover final live weight compared to *ad libitum* birds (Trocino *et al.*, 2015). In the present trial, moreover, overall feed conversion ratio was not affected by the feeding regime. Likely, the duration of the refeeding period was so long that diluted the positive effect of compensatory growth in early-restricted chickens and so short that did not allow its full expression in late-restricted chickens (25 d in the former and 11 d in the latter birds).

The effects of feed restriction on slaughter results and carcass traits are usually consistent with results of growth performance and final live weight and, accordingly, affected by rate, duration, and timing of feed restriction and refeeding. Thus, Livingston *et al.* (2018) reported lower dressing percentage and breast muscle yield in continuously restricted chickens compared to *ad libitum* fed ones. Under our conditions, the lighter late-restricted birds showed the lowest *P. major* proportion on the carcass despite no difference in dressing percentage. Gratta *et al.* (2017) also recorded lower dressing percentage and breast yield in lighter early-restricted birds compared to *ad libitum* fed chickens, consistently with literature results (Zhan *et al.*, 2007; Butzen *et al.*, 2013).

Regarding myopathies, the growth control by feed restriction was expected to have a role on the reduction of myopathy rates and degrees as well as muscle fiber degeneration at a histological level.

In fact, myopathies have been associated with an increased muscle hypertrophy of fast growing chickens which brings about reduced capillary density adjacent to the myofiber (Hoving-Bolink *et al.*, 2000; Joiner *et al.*, 2014) thus affecting regeneration, degeneration and necrosis of skeletal muscles (Velleman, 2015). Basing on gene expression and pathway by RNA-sequencing, localized hypoxia, oxidative stress, increased intracellular calcium, and the presence of muscle fiber-type switching have been proposed as responsible for wooden breast and white striping (Mutryn *et al.*, 2015; Soglia *et al.*, 2016b; Marchesi *et al.*, 2018).

Indeed, Kuttapan *et al.* (2012) reduced the occurrence of white striping in broilers slaughtered at 54 d of age by lowering the energy value of diets (75% to 53%; $P < 0.05$). Consistently, Livingston *et al.* (2018) reduced white striping (90% to 41%) and wooden breast (95% to 86%) rate as well as their degree (2.87 to 1.64 for white striping and 2.89 to 2.14 for wooden breast) in male chickens at 42 d of age. On the other hand, Meloche *et al.* (2018a) successfully reduced WS and WB severity at different ages (33, 43, and 50 d) according to feed restriction rate (from 95% to 85% of ad libitum). However, the myopathies reduction was obtained at the expenses of performance and carcass traits (Kuttappan *et al.*, 2012; Livingston *et al.*, 2018). On the other hand, and differently from the results of the present study, when early feed restriction was used to prevent myopathies and safeguard productive performance, the occurrence of white striped breasts at commercial slaughter (46 d) tended to increase (Trocino *et al.*, 2015), whereas at histological examination 97% of breasts showed MFD without significant differences between early restriction and *ad libitum* feeding (Radaelli *et al.*, 2017). These results were ascribed to the fast (muscle) growth rate of early-restricted chickens related to their compensatory growth during the refeeding period compared with birds always fed *ad libitum*.

Nevertheless, myopathies and associated MFD degeneration occur early and increase with age (14 d of age in Radaelli *et al.*, 2017; 16 d in Griffin *et al.*, 2018; 18 d in Sihvo *et al.*, 2017), as observed in the present study. Radaelli *et al.* (2017) also showed that MFD frequency and degree are under control as long and chickens are submitted to feed restriction, but the positive effects are soon mitigated after one week of refeeding to disappear completely after two weeks. The present trial confirmed that feed restriction is effective in controlling MFD during both early (13-23 d) or late restriction (27-37 d). However, nor early neither late restriction had any strong positive residual effect on myopathies rates or MFD at commercial slaughter, whereas late restriction had also negative effects on performance and carcass traits.

Consistently with our results, when the reduction of dietary lysine in different periods was used to target breast growth to control myopathy rate occurrence in male chickens, the total rate of white striped and wooden breasts was not affected (Meloche *et al.*, 2018b). Nevertheless, the occurrence of severely affected breasts decreased when the highest reduction rate was used for the longest period (i.e. 75% digestible lysine density from 18 to 26 d of age), but with detrimental effects on breast yield at 42 d of age (-1 percentage point compared to 100% digestible lysine). On the other hand, positive results on the reduction of severely affected breasts without negative effects on breast yield were obtained by a lower reduction rate (85% digestible lysine) for a longer period (12-40 d) in broiler chickens slaughtered at 61 d of age (Meloche *et al.*,

2018b). Moreover, among different nutritional strategies tested by Bodle *et al.* (2018), the decrease of amino acids density from 13 d to 24 d decreased growth in the same period, without effects on overall performance, and reduced wooden breast score in male chickens at 45 d (Bodle *et al.*, 2018). However, frequency of abnormal breasts was not significantly different between the control group and the chickens submitted to the low-amino acids dietary treatment.

The genotypes tested in our study differed each other from the the first day and until the end of the trial, with Cobb showing less growth than Ross, even if differences were greater during the second growth period. Comparison between broiler genotypes within the same trial as well as with literature data is difficult because results may be affected by several factors, like hatchery, reproductive stock age and condition, genotype nutritional requirements, and feeding plans. Nevertheless, key information may be obtained about prevalence of myopathies and interactions with growth rates (Kuttappan *et al.*, 2012; Petracci *et al.*, 2013a; Trocino *et al.*, 2015). Regarding growth performance, some authors reported better results in Cobb (500/48 Avian) *vs.* Ross 308 chickens (Hristakieva *et al.*, 2015; Benyi *et al.*, 2015). Others outlined differences between genotypes along the growth period: high-breast yield genotype performed better during the first period and worse during the second period compared to standard breast-yield chickens (Petracci *et al.*, 2013a; Trocino *et al.*, 2015) consistently with the behavior of Ross 308 and Cobb 500 chickens under the climatic conditions of the amazon region of Equador (Andrade-Yucailla *et al.*, 2017). Indeed, in our trial, Cobb also exhibited a higher mortality rate compared to Ross chickens, especially in the case of chickens always fed ad libitum or submitted to late feed restriction (significant probability of the interaction feeding system x genotype) (Table 1).

In the present study, slaughter traits and carcass quality varied between genotypes, whereas not consistent results were reported when Ross 308 and Cobb 500 results were evaluated in chickens reared under not controlled environmental conditions (Benyi *et al.*, 2015; do Nascimiento *et al.*, 2018). Regarding meat quality, few differences would have been expected between genotypes because both of them were high-growth selected hybrids. Nevertheless, meat from Ross chickens had higher pH, lower L index, higher cooking losses and shear force than meat from Cobb chickens, which could be related to the higher rate of severely white striped breasts ($P < 0.001$; Figure 1) as well as wooden breasts ($P > 0.10$; Figure 3) in the former compared to the latter group. In fact, the correlation between these changes in meat quality and myopathies occurrence has been widely demonstrated in several studies (Mudalal *et al.*, 2015; Soglia *et al.*, 2016a, 2016b; Kuttappan *et al.*, 2017).

Consistently with the present study, also Trocino *et al.* (2015) found higher rate of severely white striped breasts in high-breast yield than in standard-breast yield genotypes, without differences in the total rate of abnormal meat. Petracci *et al.* (2013b) and Lorenzi *et al.* (2014) had also found that standard genotype were less affected than high-breast-yield genotype. Indeed, in the present study, MFD degeneration score showed higher values in Ross than in Cobb at 36 d of age, when differences in growth rate between genotypes were larger, even if by slaughter age MFD was similar in the two genotypes.

Thus, whatever the feeding or nutritional strategies tested, whatever the genotype used, to our knowledge, myopathies rates are scarcely controlled, unless growth production largely impaired. In fact, MFD occurs early, it slows down when growth is reduced, but immediately restarts as soon as growth rate raises without any clear and strong beneficial residual effect at slaughter time on myopathy occurrence. At the present time, feeding strategies for growth control based on the reduction of nutrient intakes (different rates, duration, and timing) have been proved to play a partial and not always consistent control only on myopathy degree.

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CHAPTER 4: THIRD CONTRIBUTION

Effect of light management on growth, carcass and meat quality and myopathy occurrence in broiler chickens

Presented at:

TROCINO A., **GRATTA** F., BIROLO M., PICCIRILLO A., TIENGO L., XICCATO G. 2017. Myopathy occurrence and meat quality in broiler chickens: effect of genotype, gender, and light program. Proceedings of Egg&Meat2017, 03-05/09/2017, Edinburgh, UK. (oral communication), pp.40-41

Effect of light management on growth, carcass and meat quality and myopathy occurrence in broiler chickens

ABSTRACT

To reduce production costs and to maintain high profit margins, poultry industry has to look for solutions that maximize production efficiency, by using high performing genotypes and suitable farming methods.
The effects of two genotypes, of gender, and two lightening programs were evaluated on growth performance, feed conversion, slaughter results and carcass quality and meat quality of broiler chickens; moreover, the effect of white striping and wooden breast on meat quality was studied. To this purpose, 800 chicks, half Cobb 500 and half Ross 308, half males and half females, were housed in 32 collective pens at an initial stocking density of 25 birds per pen and controlled from hatching until 45 days of age. Half of the chickens were kept under a daily lighting program with 18 hours of light and 6 at dark (18L:6D); the other half under 14 hours of light and 10 hours of dark (14L:10D).
The effect of the genotype was evident since arrival: Cobb 500 chicks were heavier than Ross 308 ones (+13%; P<0.001). The difference was maintained until slaughter (+4%; P<0.001). The daily weight gain was higher in Cobb 500 chicks during the first 4 weeks, and similar in the two genotypes during the last two weeks of trial. Feed intake by pen during the whole trial was higher in Cobb than in Ross chicks (+5%; P<0.001), even if differences ranged from +12% in the first week to +7% in the fourth week to disappear by the last two weeks. The conversion index was worse in Cobb than in Ross 308 chickens (1.61 *vs.* 1.63; P<0.01). The weight of carcasses without paws was higher in Cobb than in Ross chickens (2,366 g *vs.* 2,276 g; P<0.001). At dissection, differences in carcasses weights remained but Cobb 500 showed higher proportion of whole breast (41.0% *vs.* 38.8%; P<0.001) and *Pectoralis major* muscle (12.9% *vs.* 12.0%; P<0.001). The genotype Ross showed a higher incidence of thighs than the genotype Cobb (27.9% vs 28.8%; P<0.001). The genotype did not influence the occurrence of myopathy, similar for WS (76.0% and 77.9%) and WB (6.25% and 6.25%) in the two genotypes. The genotype Ross showed higher cooking losses (27.0% vs 29.3%; P<0.001) and higher shear force (2.29 kg/g vs 2.55 kg/g; P<0.01) on the meat of *P. major* compared to genotype Cobb.
Gender also affected performance of chickens: at their arrival, females weighted 47.7 g and males 49.5 g (P<0.01); at 24 d of age, females averaged 1,081 g and males 1,235 g (P<0.001); at 45 d, females weighted 2,853 g and males 3,511 g (P<0.001). Difference of daily weight gains between females and males increased from a minimum value during the first week (-9%; P<0.001) to the highest values of the last three weeks (-27%; P<0.001). Feed intake averaged 105 g/d in female sand 125 g/d in males (P<0.001) on the whole trial, which corresponded to a worse feed conversion in females than males (1.64 vs 1.59; P<0.001). Obviously, carcasses were heavier for males than for females (weight without foots: 2580 g *vs.* 2063 g; P<0.001) as well as dressing out percentages (74.5% *vs.* 73.7%; P<0.05). Proportion of wings and tights on carcasses were similar, but females showed higher proportion of breast (40.9% *vs.* 39.0%; P<0.001) and *P. major* (25.6% *vs.* 24.2%; P<0.001) whereas they had lower proportion of hind legs than males (27.8% *vs.* 28.8%; P<0.001). The occurrence of WS (70.5% vs 83.3%) and WB (3.13% vs 9.38%) was lower (P> 0.05) in females compared to males. Gender affected many quality parameters

measured on *P. major*: the females showed lower ultimate pH values (5.92 vs 5.98; P<0.01), lower cooking losses (26.4% vs 29.9%; P<0.001) and lower shear force (2.26 kg/g vs 2.58 kg/d; P<0.01) than males; females also showed higher meat crude protein content (21.6% vs 20.7%; P<0.001) than males.

The use of different lightening programs affected live weight of chickens: at 16 d of age live weight was higher under 14L:10D than 18L:6D program (617 g vs 644 g; P<0.001). The difference of live weight was maintained until 45 d (3,130 g vs 3,233 g; P<0.001). Daily growth rate of chickens kept with 14 h of light was lower during the trial, with the exception the last week during which chickens kept with 14 h of light grew faster (-6%; P<0.001). The gap in feed consumption was relevant during the second week (62.8 g/d vs 68.1 g/d; P<0.001) but increased during the following weeks. The conversion index was better in the 14L:10D program (1.61 *vs.* 1.62; P<0.01). As for final live weight, the different lighting programs affected carcass weight at slaughter. Breast and *P. major* proportions on the whole carcass were similar, while wings (10.6% *vs.* 9.97%; P<0.01) and hind legs (28.6% *vs.* 28.0%; P<0.05) were higher in chickens kept under 14L:10D than 18L:6D. The light program 14L:10B also produced a significant reduction in the incidence of WS (from 89% to 64.6% of the controlled animals; P<0.001) and of severe WS (from 37.8% to 18.8%; P<0.01), as well as a not significant reduction of WB (from 4.17% to 8.33%; P>0.10). The light program has also affected the quality of P. major; in particular, the breast of the animals reared with 14 hours of light requested a significantly lower shear force than the chickens reared with the module 18L:6B (2.20 kg/g *vs.* 2.64 kg/g; P<0.05). The fat content was lower in animals reared with 14 hours of light (1.87% vs 2.29%; P<0.05).

The presence of myopathies changed many of the quality traits taken into account: WB breasts showed higher pH (5.92 vs 5.98; P<0.05), increased lipid content (1.82% vs 2.53; P<0.01) and lower protein content (20.9% *vs.* 20.3%; P<0.05) than normal breasts. The WB also increased cooking losses (from 27.6% to 34.2%; P<0.001) and shear force (2.90 kg/g to 3.70 kg/g; P<0.01).

In conclusion, the genotype mainly affected productive performances and carcass traits, while marginally influenced meat quality and chemical composition. The present study confirmed that for long rearing cycles males are more convenient than females in terms of productive performances and meat quality, whereas some evidences show a major incidence of meat alterations. The light program operated like a feed restriction program; the shorter daylight reduced the growth rate and final body weight, but improved feed conversion. Furthermore, this strategy successfully reduced the incidence of severe white striping, by modulating the growth rate. Finally, both abnormalities, white striping and wooden breast, had great impact on meat quality and chemical composition worsening nutritional and technological characteristics of the meat which could negatively affect consumer choice and the possibility of meat processing.

INTRODUCTION

The improving in meat production in the last years underlines the necessity to optimize productive techniques in order to obtain animal with high and standard productive performances in less time (Kuttappan *et al.*, 2016). To achieve these results the genetic companies aimed at increasing the broiler's growth rate, body weight and reducing the feed conversion (Zuidhof *et al.*, 2014). However, these rapid selection programs resulted in the occurrence of abnormalities and pathologies, e.g. leg weakness, lameness, sudden death syndrome, and myopathies (Bradshaw *et al.*, 2002). These abnormalities may worsen meat quality forcing producers to process or destroy the product due to altered aspect and technological properties (Bradshaw *et al.*, 2002; De Jong *et al.*, 2012; Petracci *et al.*, 2014b). White striping and wooden breast are the myopathies most often observed on the breast of broiler chickens characterized by different macroscopic aspect share whereas with common histological features (Kuttappan *et al.*, 2013b; Sihvo *et al.*, 2014).

Whereas the precise aetiology of these myopathies is still discussed, they are supposed to be highly linked with the growth rate of broilers (Bailey *et al.*, 2015). In order to slow down the growth rate, this way reducing the occurrence of these alterations, different strategies have been proposed. Some authors (Trocino *et al.*, 2015) proposed that genotype (high breast-yield genotype *vs.* standard yield) and gender may be involved in the occurrence and severity of myopathies. But in the years other strategies have been studied to control the growth rate of the chickens; indeed, Kuttappan *et al.* (2012c) observed a contemporary decrease in growth rate and the occurrence of WS by feeding animals with low-energy diets. However, after using early quantitative feed restriction (from 13 to 21 d of age), Trocino *et al.* (2015) did not observed any consequence on WS and WB. The authors supposed that the lack of the positive effect may be explained with the compensatory growth of the animals during the re-alimentation period.

However, in literature other feeding strategies, like schedule of photoperiod, aimed at reducing the body weight have been described. According to Classen *et al.* (1991), longer periods of darkness may limit the growth rate reducing the feed intake by preventing the regular access to feed and may be an important factor for health of broilers. However, no studies have been published on the effect of this type of management on the occurrence and severity of WS or WB.

The present study aimed at evaluating whether longer periods of darkness may affect the productive performances, meat quality, and the occurrence and severity of myopathies belonging to two genetic lines and both genders.

MATERIALS AND METODS

Description of facilities and lightening programs

The trial took place in a poultry house of the Experimental Farm "L. Toniolo" (Legnaro, Padova) belonging to University of Padova, during April and May. The poultry house had two identical rooms, with cooling system, forced ventilation, radiant heating and automatic light systems. Each room was equipped with eighteen wire-net pens (120 cm wide x 250 cm length x 120 cm height; 3.0 m^2), with a circular feeder, and 4 nipple drinkers.

For the trial, 800 broiler chickens, half belonging to Cobb 500 and half to Ross 308, were delivered by authorized truck to the experimental facility. Ross 308 chickens coming from a Italian hatchery (Montegalda, VI), while Cobb coming from a French hatchery. Before the transport all animals had been vaccinated against Marek's disease, Infectious Bronchitis, and Newcastle disease and sexed at hatchery. At the poultry house, 25 chicks per pen were randomly housed in 8 experimental group (2 photoperiod schedules, 2 genotypes, 2 genders). At the beginning of the trial all chicks were subjected to the same photoperiod schedule; from the second day of age the hours of lightness were gradually reduced from 24 h until reaching 18 hours of light and 6 hours of darkness at 9 d (standard schedule); thereafter in one room the "standard" schedule was conserved, while in the second room the light period was reduced to 16 h (at 10 d) and then to 14 h with 10 h darkness (from 11 d to 45 days) (Figure 4.1).

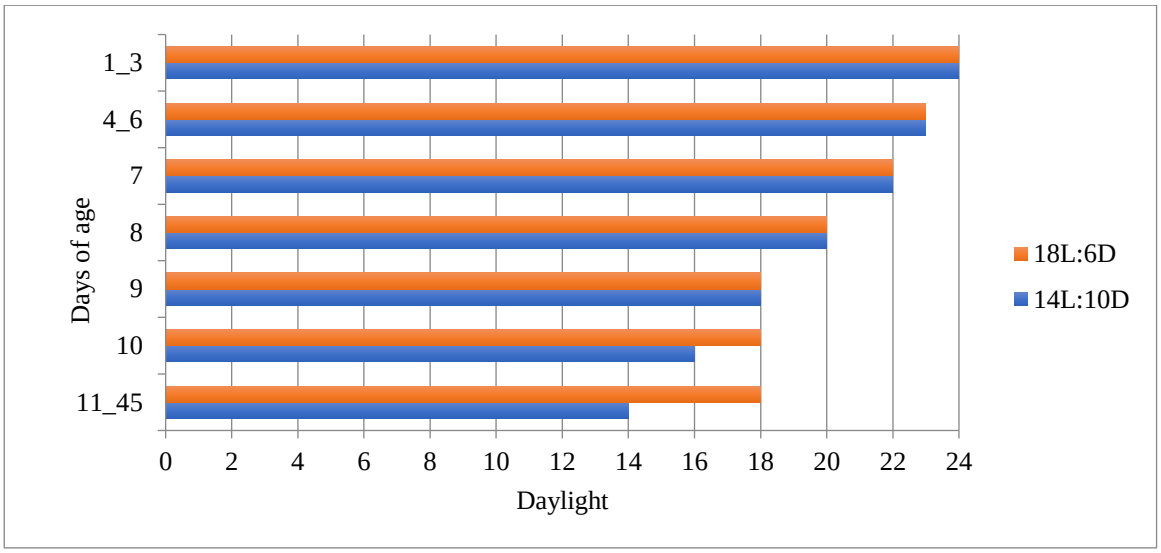

Figure 4.1 Daylight schedule in the two rooms

Commercial diets

During the trial four types of commercial diet were administrated according to the age of the animals. The first diet (MG0), fed from housing to 13 d of age, were characterized by 89% of dry matter, 23% crude

protein, 6.5% ether extract, 6% ash, and 2.6 crude fibre. From 13 d to 24 d of age, the animals were fed with the diet MG1, with similar level of dry matter, less crude protein (21.8%), higher ether extract (7.9%), lower ash (5.5%), and lower crude fibre (2.5%). In the third period, from the 24 d to 36 d of age, the diet MG2 was offered; the composition of this diet was 89.2% dry matter, 20.6% crude protein, 8.1% ether extract, 5.2% ash, and 2.5% crude fibre. In the last period, from 36 d of age until the slaughter at 45 d, the diet MG3 was administrated (dry matter 89%, crude protein 18.3%, ether extract 8.0%, ash 4.9%, and crude fibre 2.4%).

Once a week, the birds were individually weighted. Pen feed intake was measured daily with an automatic weighing system.

Health condition

Health status of birds was checked daily by clinical examination. *Post-mortem* examination of dead birds was performed to evaluate the presence and the type of pathological change. During the trial, a total of 29 animals died: 11 belonging to Ross 308 genotype and 18 belonging to Cobb 500 genotype; more precisely, 6 males and 1 female of Ross 308 and 4 males and 5 females of Cobb 500 under standard light regime (18 hours of light), and 3 males and 1 female of Ross 308 and 6 males and 3 females of Cobb 500 under reduced light regime (14 hours of light).

Commercial slaughter and analysis

At 45 d of age, the birds were individually weighted and then transported to a commercial slaughterhouse after a withdrawal of feed and water (withdrawal of 7 h for feed and 4 h for water). The animals were manually loaded on authorized truck in specific cages (83.0 cm wide x 120 cm long x 25.0 cm high; 1 m^2). Each cage contained a pen. The loading and the transport to the commercial slaughter took 1 hour and 15 min; finally, the wait until the slaughter was approximately of 3 hours. Carcasses were recovered after 2 hours of refrigeration. A total of 192 carcasses (6 per pen) had been previously selected on the basis of the slaughter live weight to be representative within a pen and were submitted to gross examination for the occurrence and severity of white striping and wooden breast on *Pectoralis major*. Afterwards, half of the carcasses (96 carcasses) were further selected for rheological and chemical analysis (Working group 5, World's Poultry Science Association, 1984). Twenty-four hours after slaughter, the breasts were submitted to meat quality analyses i.e. pH, and L*, a*, b* color indexes in triplicate on ventral surface (Sension+ portable, Hanch Company s.r.l., Swiss; Minolta Spectrophotometer CM-508 C, Minolta Corp, Ramsey, NJ, USA) (Petracci and Baéza, 2011). After that, a portion of *P. major* (8 cm x 4 cm x 3 cm) was separated from the rest of the meat to be submitted to chemical analyses according to AOAC methods (2000). The meat portion was weighted and stored at -18°C until meat analyses.

After 10 days, the meat portions were recovered to measure thawing losses. The thawed sample was put under vacuum in plastic bags and cooked in a water bath for 45 minutes, to reach internal temperature of 80°C. After cooling, the cooking losses were measured and a smaller meat portion was obtained from the cooked sample (4 cm x 2 cm x 1 cm) to measure the shear force using a LS5 dynamometer (Lloyd Instruments Ltd, Bognor Regis, UK) with the Allo-Kramer probe (10 blades) (load cell: 500 kg; distance between the blades: 5 mm; thickness: 2 mm; cutting speed: 250 mm/min) (Mudalal *et al.*, 2015).

Statistical analysis

Individual data of initial and final weights, daily growth, slaughter results, and carcass and meat traits were analysedby ANOVA with photoperiod, genotype, gender, and their interaction as main factors of variability and with pen as a random effect, by the PROC MIXED of SAS software (SAS Institute Inc., 2011). Cage data for feed intake and feed conversion were analysed by ANOVA with photoperiod, genotype, gender, and their interactions as main factors of variability, and by the PROC GLM (SAS Institute Inc., 2011). When necessary, the Bonferroni *t* test was used to compare least squares means. The frequency of chickens showing myopathies at *P. major* was analysed by PROC CATMOD (SAS Institute Inc., 2009) according to photoperiod, genotype, gender. Thereafter, differences according to the feeding system within age were assessed by the χ^2 test.

RESULTS AND DISCUSSION

During the trial the effect of genotype resulted significant since the first day: at 1 d of age the chicks of Cobb 500 were heavier (+13%, P<0.001) respect to the Ross 308 ones and after two days the difference between the two genotypes increased (+16%, P<0.001). However during the experiment, the differences decreased but still remain significant; indeed, at 23 d of age, Cobb chickens weighted +6% of Ross ones, and at the end of the trial the difference was reduced at +4% (P<0.001) (Table 4.1). These differences in live weight derived from significantly higher daily growth in the Cobb animals respect to the Ross until the fourth week, while the last chickens grew faster in the last week. During whole trial the growth rate resulted higher in Cobb than in Ross chickens (72.5 g/d *vs.* 69.9 g/d; P<0.001). Also feed intake appeared higher in Cobb genotype (+5%; P<0.001), whereas feed conversion resulted worse in Cobb 500 (1.63 *vs.* 1.61; P<0.01). In literature, other authors (Hristakieva *et al.*, 2014) referred better productive performances for Cobb 500 than Ross ones reporting heavier weights at hatchery(P<0.05) and significant differences at the end of the trial (+6.29%); However, it is important to underline the effect of the weight at hatchery because it could affect the productive performances. Differently to Hristakieva *et al.* (2014), our animals came from different hatcheries and the Cobb ones were submitted to a longer transport.

The effect of the gender was relevant since the arrival of the chicks to poultry house and the difference in live weight between males and females increased with age. At the arrival females weighted 47.7 g respect to 49.5 g of males (P<0.01); at 24 d of age females were still lighter than the other gender (-12.5%; P<0.001); while at end of the trial the weight of female gender was 2,853 g *vs.* 3,511 g of males (P<0.001). These differences in live weight are the result of different growth rate, always higher in males; the gap in growth rate increased from the first week (+9%; P<0.001) until the last day of trial (+27%; P<0.001). Feed intake was lower during all the weeks for the female gender, with higher differences during the fourth (125 g/d *vs.* 155 g/d; P<0.001) and fifth week (153 *vs.* 190 g/d; P<0.001); referring to the whole trial the females had an average feed intake of 105 g/d and the males of 125 g/d (P<0.001). Finally, the feed conversion appeared to be significantly higher in female animals (1.64 *vs.* 1.59; P<0.001). Similarly, Hristakieva *et al.* (2014) observed that males were more performant than females. Benyi *et al.* (2015) also described some significant interactions of sex and genotype on productive performances.

The photoperiod program impaired the live weight of the animals; indeed since 9 d of age, which is the day of the change in the photoperiod schedule in the two rooms, the chickens submitted to 18 hours of light were heavier respect to the chicks submitted to 14 hours of light (644 g *vs.* 617 g; P<0.001). the difference in live weight was maintained until the end of the trial: chickens reared under the 14L photoperiod resulted lighter than animals reared with 18L period (3,130 g *vs* 3,233 g; P<0.001). The growth rate increased according to the live weight of animals submitted to different light regime: animals with shorter daylight could ate less and thus, grow less, especially on the third week (-9%; P<0.001); however, in the last week of trial the

animals with shorter daylight were characterized by higher growth rate (+6%; P<0.001), whereas, on the whole trial the growth rate was better for animals with longer photoperiod (+3%; P<0.001) (Table 4.1). The feed intake, according to growth rate, was similar until the photoperiod was the same, then, at the second week started to be significantly different (. 62.8 g/d *vs* 68.1 g/d respectively for 14L and 18L; P<0.001) and remained significant until the last week of trial (177 g/d *vs*181 g/d; P<0.05). However, the feed conversion appeared to be worse for animals submitted to longer photoperiod, despite the differences from numerical point of view were quite marginal (1.61*vs.*1.62; P<0.01). So, the reduction in daylight could be rightfully considered an indirect feeding restriction program. According to some authors, the worsening of productive performances due to the feed restriction depends on various factors, like the duration of these strategies and the re-alimentation period, in which animals submitted to restriction undergo to compensatory growth (Sahraei, 2012; Trocino *et al.*, 2015). Moreover, Trocino *et al.* (2015) reported that animals submitted to early feed restriction were lighter than animals fed *ad libitum* (-2%) besides the compensatory growth; on the other hand, the restricted animals showed a better feed conversion than *ad libitum* chickens. In the present study, a certain recovery on performances of the animals submitted a shorter daylight happened. As said above, from the second week of rearing, the 14L group showed a reduction in consumption, 8%; although this difference decreased with the increasing of the age (7%, 4%, 4%, 2%, in the third, fourth, fifth, and sixth week respectively) respect to 18L photoperiod, mitigating in this way the gap between the two treatment.

Table 4.1 Effect of genotype, gender, and photoperiod on productive performances (individual data)

	Genotype (G)		Gender (S)		Daylighr (L)		Prob						MSE
	Cobb 500	Ross 308	F	M	14L	18L	G	S	L	GxS	GxL	L×S	
Chickens, n	329	331	330	330	330	330							
Weight, g													
0 d	51.6	45.6	47.7	49.5	48.4	48.7	<0.001	<0.01	0.61	<0.01	0.53	0.85	3.75
3 d	73.2	63.3	66.7	69.8	68.3	68.2	<0.001	<0.001	0.87	<0.001	0.41	0.77	5.55
10 d	284	255	259	280	271	269	<0.001	<0.001	0.63	<0.001	0.87	0.81	22.9
17 d	650	612	599	663	617	644	<0.001	<0.001	<0.001	<0.001	0.94	0.90	43.5
24 d	1,192	1,124	1,081	1,235	1,122	1,194	<0.001	<0.001	<0.001	<0.01	0.61	0.70	73.2
31 d	1,886	1,780	1,667	1,999	1,785	1,881	<0.001	<0.001	<0.001	<0.05	0.38	0.87	118
38 d	2,631	2,510	2,316	2,826	2,502	2,640	<0.001	<0.001	<0.001	0.11	0.37	0.54	174
45 d	3,242	3,122	2,853	3,511	3,130	3,233	<0.001	<0.001	<0.001	0.09	0.79	0.89	215
Growth rate, g/d													
Week 1[1]	25.9	23.3	23.5	25.6	24.7	24.5	<0.001	<0.001	0.55	0.99	<0.001	0.81	2.38
Week 2	52.2	51.0	48.5	54.7	49.5	53.6	0.07	<0.001	<0.001	0.83	<0.01	0.87	4.40
Week 3	77.5	73.1	68.8	81.8	72.1	78.5	<0.001	<0.001	<0.001	0.32	0.12	0.32	5.80
Week 4	99.1	93.7	83.6	109	94.7	98.1	<0.001	<0.001	<0.05	0.24	0.37	0.92	8.92
Week 5	106	104	92.9	118	102	109	0.09	<0.001	<0.001	0.60	0.31	0.26	11.5
Week 6	93.9	94.1	82.7	105	96.7	91.3	0.92	<0.001	<0.001	0.07	0.53	<0.05	13.5
Whole trial	72.5	69.9	63.8	78.7	70.0	72.4	<0.001	<0.001	<0.001	0.80	0.11	0.89	4.88

1: from day 1 until day 10

Table 4.2 Effect of genotype, gender, and photoperiod on productive performances (pens data)

	Genotype (G)		Gender (S)		Daylight (L)		Prob						MSE
	Cobb 500	Ross 308	F	M	14L	18L	G	S	L	GxS	GxL	L×S	
Pen, n	16	16	16	16	16	16							
Growth rate													
Week 1[1]	25.9	23.3	23.5	25.7	24.7	24.5	<0.001	<0.001	0.59	<0.001	0.95	0.58	0.90
Week 2	52.2	51.0	48.4	54.8	49.5	53.6	0.10	<0.001	<0.001	<0.01	0.87	0.93	1.89
Week 3	77.5	73.2	68.7	82.0	72.1	78.6	<0.01	<0.001	<0.001	0.10	0.31	0.26	3.29
Week 4	99.1	93.8	83.5	109	94.7	98.2	<0.01	<0.001	<0.05	0.32	0.24	0.98	4.51
Week 5	106	104	93.0	118	102	109	0.12	<0.001	<0.001	0.30	0.57	0.31	3.63
Week 6	93.9	94.1	82.7	105	96.7	91.4	0.90	<0.001	<0.01	0.53	0.09	<0.05	4.13
Whole trial	72.5	70.0	63.7	78.7	70.0	72.4	<0.01	<0.001	<0.01	0.11	0.75	0.98	2.00
Feed intake, g/d													
Week 1[1]	29.2	26.0	26.5	28.7	27.5	27.7	<0.001	<0.001	0.60	<0.01	0.23	0.26	1.33
Week 2	66.2	64.7	62.7	68.2	62.8	68.1	0.16	<0.001	<0.001	<0.01	0.81	0.86	2.76
Week 3	111	102	99	114	103	110	<0.001	<0.001	<0.001	<0.05	0.24	0.57	4.40
Week 4	145	135	125	155	137	143	<0.001	<0.001	<0.01	0.12	0.53	0.41	5.89
Week 5	175	167	153	190	168	174	<0.001	<0.001	<0.01	0.99	0.94	0.67	5.58
Week 6	180	178	162	195	177	181	0.31	<0.001	<0.05	0.43	0.22	0.68	5.79
Whole trial	118	112	105	125	112	117	<0.001	<0.001	<0.001	0.10	0.50	0.83	3.56
Feed conversion													
Week 1[1]	1.13	1.12	1.13	1.12	1.12	1.13	0.46	0.37	0.25	0.08	0.10	0.34	0.04
Week 2	1.27	1.27	1.30	1.24	1.27	1.27	0.93	<0.01	0.78	0.67	0.98	0.81	0.05
Week 3	1.44	1.40	1.44	1.39	1.43	1.41	<0.01	<0.001	<0.05	0.25	0.70	0.38	0.03
Week 4	1.47	1.45	1.50	1.42	1.45	1.46	<0.05	<0.001	0.35	0.21	0.09	<0.05	0.02
Week 5	1.65	1.60	1.64	1.61	1.64	1.61	<0.01	<0.05	<0.05	0.08	0.66	0.11	0.04
Week 6	1.93	1.90	1.97	1.86	1.84	1.99	0.29	<0.001	<0.001	0.78	<0.01	0.11	0.06
Whole trial	1.63	1.61	1.64	1.59	1.61	1.62	<0.01	<0.001	<0.01	0.43	0.13	0.57	0.02

1: from day 1 until day 10

The effect of genotype was evident also on carcass traits (Table 4.3). The carcasses with paws belonging to Cobb 500 were heavier than Ross ones (2,471 g *vs.* 2,379 g; P<0.001) and also carcasses without paws (2,366 g *vs.* 2,276 g; P<0.001). The medium dressing out percentage was around the 74%. The carcasses selected for the further meat quality analyses were representative of two genotypes with medium weight of 2,279 g and 2,162 g (P<0.001). The breast yield resulted higher in Cobb genotype either in whole breast (41.0% *vs.* 38.8%; P<0.001) or *P.major* (12.9% *vs.* 12.0%; P<0.001). Differently, wings (10.1% *vs.* 10.5%; P<0.001), drumstick (13.0% *vs.* 13.4%; P<0.001), thigs (14.8% *vs.* 15.4%; P<0.05) resulted lower in Cobb than Ross. Higher breast yield, and lower incidence of hind leg of Cobb genotype respect to Ross ones was already described by Hristakieva *et al.* (2014). Differently, Janish *et al.* (2011), comparing different Ross and Cobb genotypes (Ross 308, Ross 708, Cobb 700) slaughtered at different ages, did not observed significant variations on the carcass quality.The effect of the genotype on the meat quality was limited to cooking losses and to shear force. The Cobb 500 meat had less loss during the cooking phase (-7.8%; P<0.001) and was more tender than Ross308 meat (2.29 kg/g *vs.* 2.55 kg/g; P<0.01). On the other hand, the variations of the meat chemical composition due to the genotype are few (ash) and with neglectable meaning.The effect of gender was important on all carcass traits. The huge difference in live body weight of the two genders, higher in males than in females (3,439 g *vs.* 2790 g; P<0.001), resulted in heavier carcasses (2,580 g *vs.* 2,063 g; P<0.001). Moreover, the males had better carcass yield either in carcass with paws (78.0% *vs.* 76.7%; P<0.001) or without paws (74.5% *vs.* 73.7%; P<0.05). The incidence of wings and drumstick are quite similar, while we observed higher values for hind leg (28.8% *vs.* 27.8%; P<0.001) breast yield (40.9% *vs.* 39.0%; P<0.001) and incidence of *P.major* (12.8% *vs.* 12.1%; P<0.001) in males than females. Other authors detected similar differences on carcass quality due to gender (Abdullah *et al.*, 2010; Baeza *et al.*, 2010; Hristakieva *et al.*, 2014). Similarly, the effect exerted by the gender on meat quality was great. The gender affected either meat quality or the meat chemical composition: cooking losses were significantly higher in males than in females (26.4% *vs.* 29.9%; P<0.001), also males had harder meat than females (2.26 *vs.* 2.58; P<0.01). For what concern the chemical composition, males had significantly higher content of water (75.9% *vs.* 75.1%; P<0.001) and lower content of ash (1,07% *vs.* 1.08%; P<0.05) and crude protein (20.7% *vs.* 21.6%; P<0.001). In contrast, none effect of gender was observed on ether extract (Table 4.4).The photoperiod affected significantly the slaughter results and carcass quality. The animals submitted to a longer photoperiod (18 hours of light) showed higher weight at slaughter (+6%; P<0.001), and higher carcass weight (+6%; P<0.001). However, the dressing out percentage in two groups resulted similar, around 74%. The incidence of cut resulted similar for breast yield and *P. major*, while the incidence of wings and hind legs resulted lower in animal submitted to a longer daylight, respectively 9.97% *vs* 10.6% (P<0.01) and 28.0% *vs.* 28.6% (P<0.05) (Table 4.3). Whereas the photoperiod affected marginally meat quality, chickens submitted to shorter daylight had more tender meat (2.20 kg/g *vs.* 2.64 kg/g; P<0.05), it largely affected the chemical composition; indeed, meat of animal with longer darkness period had higher content of water (75.7% *vs.* 75.3%; P<0.05), lower content of ash (1.06% *vs.* 1.09%; P<0.05) and ether extract (1.87% *vs.* 2.29%; P<0.05) while the crude protein was unchanged (Table 4.4).

Table 4.3 Effect of genotype, gender, and photoperiod on carcass traits

	Genotype (G)		Gender (S)		Daylight(L)		Prob						MSE
	Cobb 500	Ross 308	F	M	14L	18L	G	S	L	GxS	GxL	L×S	
Chickens, n	96	95	95	96	96	95							
Weight at slaughter, g	3,184	3,046	2,790	3,439	3,018	3,212	<0.001	<0.001	<0.001	0.30	0.56	0.98	216
Chilled carcass 3 h, g	2,366	2,276	2,063	2,580	2,247	2,396	<0.001	<0.001	<0.001	0.19	0.76	0.80	166
Dressing out 3 h, %	74.0	74.2	73.7	74.5	73.9	74.2	0.53	<0.05	0.28	0.20	0.89	0.67	1.49
Chickens, n	48	48	48	48	48	48							
Chilled carcass 24 h (CC), g	2,279	2,162	1,967	2,474	2,107	2,335	<0.001	<0.001	<0.001	0.21	0.84	0.87	164
Dressing out 24 h, %	73.6	73.7	73.2	74.1	73.5	73.8	0.62	<0.01	0.31	0.21	0.97	0.68	1.50
Breast, % CC	41.0	38.8	40.9	39.0	39.8	40.0	<0.001	<0.001	0.72	0.21	0.29	<0.05	2.08
Wings, % CC	10.1	10.5	10.3	10.3	10.6	9.97	<0.001	0.93	<0.01	0.82	0.23	0.60	0.47
Drumstick, % CC	13.0	13.4	12.9	13.5	13.2	13.2	<0.01	<0.001	0.95	0.90	0.24	0.45	0.72
Thighs, % CC	14.8	15.4	15.0	15.3	15.4	14.8	<0.05	0.14	<0.01	0.41	0.62	0.87	1.02
Hind legs, % CC	27.8	28.8	27.9	28.8	28.6	28.0	<0.001	<0.001	<0.05	0.49	0.80	0.60	1.35
Pectoralis major, % CC	25.8	24.0	25.6	24.2	24.6	25.2	<0.001	<0.001	0.32	0.30	0.30	<0.05	0.89

MSE, root mean square error; SEM is equal to MSE/√n.

Table 4.4 Effect of genotype, gender, photoperiod on meat quality and chemical composition

	Genotype (G)		Gender (S)		Daylight (L)		Prob						MSE
	Cobb 500	Ross 308	F	M	14L	18L	G	S	L	GxS	GxL	L×S	
Chickens, n	48	48	48	48	48	48							
pH	5.94	5.95	5.92	5.98	5.95	5.94	0.32	<0.01	0.84	0.48	0.19	0.94	0.11
L*	45.9	46.3	46.3	46.0	46.0	46.2	0.34	0.46	0.72	0.56	<0.01	<0.05	2.01
a*	-0.08	-0.08	-0.02	-0.15	-0.18	0.02	0.99	0.25	0.08	<0.05	0.10	0.77	0.54
b*	15.6	15.8	15.7	15.6	15.7	15.6	0.65	0.77	0.89	0.22	0.11	0.43	2.10
Thawing loss, %	6.44	6.40	6.34	6.51	6.31	6.54	0.92	0.68	0.76	0.90	0.24	0.30	1.89
Cooking loss, %	27.0	29.3	26.4	29.9	28.0	28.3	<0.001	<0.001	0.73	0.77	0.88	0.59	3.19
Shear force, kg/g	2.29	2.55	2.26	2.58	2.20	2.64	<0.01	<0.01	<0.05	0.71	0.55	0.29	0.46
Chemical composition													
Water, %	75.5	75.5	75.1	75.9	75.7	75.3	0.97	<0.001	<0.05	0.88	0.95	0.50	0.77
Ash, %	1.08	1.07	1.08	1.07	1.06	1.09	<0.05	<0.05	<0.05	0.06	0.22	0.77	0.03
Crude protein, %	21.3	21.0	21.6	20.7	21.1	21.2	0.06	<0.001	0.47	0.74	0.75	0.24	0.87
Ether extract, %	1.94	2.22	1.94	2.22	1.87	2.29	0.06	0.06	<0.05	0.42	0.65	0.56	0.72

MSE, root mean square error; SEM is equal to MSE/√n.

Concerning the occurrence of myopathies, during the experimental trial the incidence of these alterations was quite elevated in the case of WS, around 77%, while the incidence of WB was lower compared to the previous described myopathy, around the 6.25%. Trocino *et al.* (2015) found similar incidence of WS (74.5%) while reported a higher occurrence of WB (24.3%).

Many studies showed that the occurrence of WS is higher in genetic strains selected for high breast yield (Kuttappan *et al.*, 2013b; Lorenzi *et al.*, 2014); on the other hand, in the present and in previous trials (Trocino *et al.*, 2015) no significant differences in the occurrence of these abnormalities were observed in the investigated genotypes (Table 4.5). According to Bailey *et al.* (2015), the heritability of these alterations is quite low, while the environmental factors play a central role in the myopathy development.

As for the effect of genotype, the gender did not affect the occurrence of WS, even though from numerical point of view the incidence resulted higher in males than in females, similarly to what observed by Kuttappan *et al.* (2013b).

On the contrary, the reduction of the daylight, 14 hours of light, resulted in the reduction of WS occurrence (64.6% *vs.* 89.5%; $P<0.001$). This reduction was also accompanied by the reduction of WS severity (18.8% *vs.* 37.9%; $P<0.01$) (Table 4.5).

Finally, the light programs and gender affected also WB incidence. Indeed, this alteration was mostly observed in animal submitted to longer daylight than animals with a shorter daylight (8.33% *vs.* 4.17%; $P>0.10$) and in males than females (9.38% *vs.* 3.13%; $P=0.11$) even though these effects can not statistically confirm due to limited number of breasts affected by this alteration.

Table 4.5 Effect of genotype, gender, photoperiod on myopathy occurrence and severity of white striping.

	Genotype (T)		Gender (S)		Daylight (L)		Prob					
	Cobb 500	Cobb 500	F	M	14L	18L	G	S	L	TxL	TxS	L×S
Chickens, n	96	95	95	96	96	95						
White striping WS, %	76.0	77.9	70.5	83.3	64.6	89.5	0.80	0.80	<0.001	0.52	<0.05	<0.05
Wooden breast WB, %	6.25	6.25	3.13	9.38	4.17	8.33	0.75	0.75	0.31	0.99	0.50	0.98
WS severity												
1 (mild), %	47.9	49.5	44.2	53.1	45.8	51.6	0.87	0.87	0.42	0.80	<0.05	0.09
2 (severe), %	28.1	28.4	26.3	30.2	18.8	37.9	0.76	0.76	<0.01	0.47	0.27	0.35

MSE, root mean square error; SEM is equal to MSE/√n.

Table 4.6 Effects of the presence of myopathies on the quality of *Pectoralis major*

	White Striping (WS)		Wooden Breast (WB)		Prob		MSE
	No	Yes	No	Yes	WS	WB	
Chickens, n	44	147	180	12			
pH	5.92	5.98	5.92	5.98	<0.05	0.23	0.11
L*	46.4	46.0	46.2	46.1	0.53	0.90	2.15
a*	-0.32	-0.26	-0.08	-0.50	0.70	0.11	0.56
b*	14.6	14.9	15.6	13.8	0.55	<0.05	2.08
Thawing loss, %	8.22	8.40	8.06	8.56	0.76	0.60	2.08
Cooking loss, %	30.2	31.6	27.6	34.2	0.12	<0.001	3.43
Shear force, kg/g	3.21	3.39	2.90	3.70	0.19	<0.01	0.51
Chemical composition,							
Water, %	76.0	75.9	75.6	76.3	0.97	0.07	0.87
Ash, %	1.10	1.08	1.11	1.07	<0.05	<0.01	0.03
Crude protein, %	20.9	20.3	21.4	19.8	<0.05	<0.001	0.90
Ether extract, %	1.82	2.53	1.75	2.60	<0.001	<0.01	0.69

MSE, root mean square error; SEM is equal to MSE/√n.

The presence of WS had a little impact on the meat rheological traits, affecting only the pH of the meat; indeed, normal fillets have lower pH respect to white striping ones (5.92 *vs.* 5.98; P<0.05). On the other hand, the presence of this alterations changed the chemical composition the meat: normal meat had higher content of ash (1.10% *vs.*1.08%; P<0.05), crude protein (20.9% *vs.* 20.3%; P<0.05), and lower content of ether extract (1.82% *vs.* 2.53%; P<0.001). Differently, WB significantly changed either rheological quality and chemical composition: a significant reduction in yellow index (15.6 *vs.* 13.8; P<0.05) and increase of cooking loss (27.6% *vs.* 34.2%; P<0.001) and shear force (2.90 kg/g *vs.* 3.70) were measured. This myopathy changed also the meat chemical composition, decreasing the percentage of ash (1.11% *vs.* 1.07%; P<0.01) and crude protein (21.4% *vs.* 19.8%; P<0.001) and increasing ether extract (1.75% *vs.* 2.60; P<0.01) (Table 4.6).

Analysing the severity of the WS, we observed that normal meat had lower pH respect to meat with a severe degree of WS, while mild level of WS shoed an intermediate value (5.89 *vs.* 5.94 *vs.* 5.99; P<0.01) (Table 4.7). In similar way, the content of ash, crude protein, and ether extract changed according to WS degrees. In normal fillets, the content of lipids was around 1.39% and increased up to 2.56% in severe WS (P<0.001); similarly changed the content of ash and crude protein (P<0.05). Also, the cooking loss showed a tendency to increase according to white striping degrees.

Table 4.7 Effect of white striping degree on meat traits and chemical composition

	White striping degree			Prob	MSE
	0	1	2		
Chickens., n	44	93	54		
pH	5.89^{a}	5.94^{ab}	5.99^{b}	<0.01	0.10
L*	46.4	46.1	46.0	0.81	2.15
a*	-0.11	-0.02	-0.19	0.45	0.57
b*	15.5	16.0	15.1	0.23	2.09
Thawing loss, %	7.97	8.37	7.80	0.48	2.07
Cooking loss, %	26.9^{a}	28.4^{ab}	29.4^{b}	0.10	3.72
Shear force, kg/g	2.81	3.03	3.07	0.27	0.54
Chemical composition,					
H_2O, %	75.6	75.8	75.3	0.15	0.87
Ash, %	1.12^{b}	1.11^{ab}	1.10^{a}	<0.05	0.03
Crude protein, %	21.7^{b}	21.1^{ab}	20.8^{a}	<0.05	0.96
Ether extract, %	1.39^{a}	1.96^{b}	2.56^{c}	<0.001	0.66

MSE, root mean square error; SEM is equal to MSE/√n.

The presence of these alterations not only results in worsening of macroscopic characteristics, but also in worsening in meat quality. Different authors refer of an increase in pH and in worsening of chemical composition of affected meat. In particular, Petracci *et al.* (2014b) reported an increase of lipids in WS meat.

Similarly, WB meat, besides an altered chemical composition, shows a worsening in technological characteristics. Indeed, Trocino *et al. (2015)* observed an increase in cooking loss (+19.3%), and in shear

force (+21.6%) in presence of WB. Other authors (Tasoniero *et al.*, 2016) affirm that the simultaneous presence of WS and WB could alter even more chemical and technological traits.

In conclusion, the two genotypes used in our trial showed different results on productive performances; the hybrid Cobb 500 had higher final weight and growth rate, whereas Ross 308 showed better feed conversion. The effect of gender was clear and confirmed that males are more performant with high growth rate and better carcass traits. The two lightening programs resulted in different productive performances and quality traits. The animals submitted to a shorter daylight had lower growth rate and live body weight. However, the reduction of light duration improved feed conversion and significantly reduced the occurrence of WS.

On the other hand, the occurrence of WS and WB had repercussions on meat quality: WS meat had higher pH and low nutritional value (high lipids and low protein), while WB impaired both technological traits and chemical composition.

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CHAPTER 5: FOURTH CONTRIBUTION

Effect of breast myopathies on quality and microbial shelf life of broiler meat

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ABSTRACT

To evaluate the impact of emerging myopathies on meat quality and microbial shelf life, 48 normal, 48 white striped (WS), and 48 wooden (WB) breasts were stored during 11 d at 4°C using PVC film and analysed at 24, 72, 120, 168, 216, and 264 h post-mortem. Overall, normal breasts showed lower ($P < 0.001$) redness index (−0.88 *vs.* −0.41 and −0.43) and cooking losses (22.0% *vs.* 23.8% *vs.* 26.9%) than WS and WB. Then, normal and WS breasts exhibited higher protein content than WB (23.9% and 23.2% *vs.* 21.4%; $P < 0.001$). Normal meat had also lower ether extract content than WB (1.09% *vs.* 1.88%; $P < 0.001$) with intermediate values for WS. Normal breasts exhibited higher saturated fatty acids (FA) (31.3% *vs.* 28.0% on average) and lower unsaturated FA rates (68.7% *vs.* 72.0%) than WS and WB ($P < 0.001$). Differences were mainly to polyunsaturated FA (30.5% in normal *vs.* 35.3% and 35.4% in WS and WB; $P < 0.001$). During storage, technological and chemical traits of normal and affected meat showed a similar pattern. Nevertheless, normal breasts had the highest initial total viable count (TVC) and the shortest TVC lag phase than WS and WB (46.3 h *vs.* 85.2 h and 77.8 h). Microbial shelf life threshold (7 $\log_{10}$ CFU TVC/g) was achieved first in normal (130 h) than in WS (149 h) and WB (192 h). Besides, TVC and *Pseudomonas* spp. counts were significantly higher in normal that affected breasts between 72 h and 216 h of storage. *Enterobacteriaceae* spp. and LAB counts were significantly higher in normal meat, lower in WB, and intermediate in WS since the first sampling, after 24 h, and until 216 h. All differences in microbial targets across meat types disappeared by 264 h of storage (i.e. 11 d). Further studies are necessary to elucidate the factors and the mechanisms that may modulate microbial growth and composition during storage in broiler breast meat affected by myopathies.

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INTRODUCTION

Poultry market has changed a lot during the last 50 years. The high consumer acceptability of poultry meat for its healthy perceived value and little culinary skill needs, as well as suitability for further processing, have increased the poultry demand (Petracci *et al.*, 2013a). This increase has thus driven producers to optimize farming techniques to produce more in less time (Kuttappan *et al.*, 2016). Besides, the increased consumer awareness for food safety has driven poultry industry to guarantee high meat quality standards (Mead, 2004; Grashorn, 2017).

On the other hand, poultry industry is facing the raising occurrence of meat abnormalities due to both pre-slaughter and slaughtering factors (Kuttappan *et al.*, 2016). Some of these defects have been also observed in other species (e.g. pale soft and exudative meat in pork) and largely described in literature. Other abnormalities are specific of poultry, such as white striping (**WS**) and wooden breast (**WB**) affecting *Pectoralis major* muscle of broiler breast (Kuttappan *et al.*, 2013 and 2016; Trocino *et al.*, 2015; Tijare *et al.*, 2016). These defects do not only alter the meat visual appearance, but also modify the muscle chemical composition and histological traits: both WS and WB increase intramuscular fat content and decrease protein, and provoke some dysfunctions at muscle tissues, with the consequent degeneration of myofibrillar structure (Mudalal *et al.*, 2015; Soglia *et al.*, 2017; Radaelli *et al.*, 2017). Consequently, these myopathies may affect features influencing consumers' judgment (texture, juiciness, tenderness, color, flavor) (Kuttappan *et al.*, 2012b; Tasoniero *et al.*, 2016) as well as technological properties of meat (pH, water holding and binding capacity, texture) (Mudalal *et al.*, 2015; Trocino *et al.*, 2015; Soglia *et al.*, 2016a).

Numerous studies discuss about mechanisms involved in meat changes due to WS and WB (Kuttappan *et al.*, 2016). However, quite little is known about quality behavior of these meats during storage (Soglia *et al.*, 2017; Sun *et al.* 2018). Besides, the presence of pathogenic and spoilage microorganisms in abnormal meat have been little investigated, and, to our knowledge, only one study has assessed microbial growth in WB breasts (Dalgaard *et al.*, 2018). This issue would be an additional significant concern to poultry industry, due to the possible effects on microbial shelf life and food safety of meat both sold fresh and used for processing. Thus, the present study aimed at comparing technological, chemical, and microbiological traits as well as microbial shelf life during storage for 11 d in normal, WS, and WB broiler breast meat.

MATERIALS AND METHODS

Sampling procedure

At a commercial slaughterhouse, 144 carcasses were selected from a single batch of 10,401 male chickens, belonging to Ross 308 genotype, slaughtered at 49 d of age, and at an average slaughter BW of 2,797 g. After the chilling tunnel, a trained and skilled operator selected 48 normal breasts, 48 white striped breasts, and 48 wooden breasts, according to the criteria proposed by Kuttappan *et al.* (2012b) and Sihvo *et al.* (2014).

Skinless bone-in breasts were then individually numbered and packed using rigid trays and PVC film, and transported in a refrigerated truck at 4°C to the Department laboratories within 24 h after slaughter. At the lab, the trays were stored in a refrigerated cabinet (Costan SpA, Belluno, Italy) for 264 h (corresponding to 11 d). Storage setting was designed to reproduce the standard refrigeration condition during sale (packaging and exposure to light). Exposure to light (OSRAM L36W/76-1) was set from 8:00 to 20:00 at an average temperature of 4±1°C.

Breasts were controlled at 24, 72, 120, 168, 216, and 264 h (corresponding to 1, 3, 5, 7, 9, and 11 d) after slaughter for meat technological, chemical, and microbiological traits. At each sampling day, 24 breasts were processed (8 normal, 8 WS, and 8 WB). First, the presence of white striping and wooden breast was confirmed at gross examination. Then, breasts were dissected into the right and the left *Pectoralis major* by sterile tools. The right *Pectoralis major* muscles were processed for microbiological analyses. The left ones were used for all other analyses.

Technological analyses

At each sampling day, left *Pectoralis major* muscles were submitted to meat quality analyses according to Petracci and Baéza (2011). The pH was measured in triplicate on the muscle ventral side with a pH-meter equipped with a specific electrode for meat penetration and a probe with automatic temperature control (portable Sension+, Hach, Geneva, Switzerland). The L*a*b* color indexes were measured in triplicate on the ventral side of the same muscles, using a Minolta CM-508 C spectrophotometer (Minolta Corp., Ramsey, NJ, USA).

Thereafter, a parallelepiped meat portion (8 cm × 4 cm × 3 cm), parallel to muscle fibres directions, was separated from the cranial side of *Pectoralis major* and stored under vacuum in plastic bags at -18°C until meat analyses. Thawing and cooking losses were measured in this cut according to Petracci and Baéza (2011). After thawing, the meat portion was put into plastic bags and cooked in a water bath for 45 min until an internal temperature of 80°C was achieved. After a 40-min cooling, a further parallelepiped meat

portion (4 cm × 2 cm × 1 cm) was separated from the original one. On this latter section, the maximum shear force was measured with LS5 dynamometer (Lloyd Instruments Ltd, Bognor Regis, UK) using the Allo-Kramer probe (10 blades; load cell: 500 kg; distance between the blades: 5 mm; blade thickness: 2 mm; cutting speed: 250 mm/min) (Mudalal *et al.*, 2015).

Microbiological analyses

At each sampling day, a total of 15 right *Pectoralis major* muscles (5 normal, 5 WS, and 5 WB) were submitted to microbiological analyses. A 25-g sample was homogenized in a sterile stomacher bag with 225 mL of buffered peptone water followed by serial dilutions. Total viable count (**TVC**) was evaluated on Plate Count Agar (Biokar Diagnostics, Beauvais, France) incubated at 30°C for 72 h. The contamination provided by *Enterobacteriaceae* was assessed by Violet Red Bile Glucose Agar (Biokar Diagnostics, Beauvais, France) incubated at 37°C for 24 h. Lactic acid bacteria (**LAB**) were analysed on De Man, Rogosa and Sharpe Agar (Biokar Diagnostics, Beauvais, France) in anaerobic conditions at 30°C for 48 h. *Pseudomonas* spp. count was evaluated on *Pseudomonas* Agar Base supplemented with cetrimide, fucidine, cephaloridine at 25°C for 48 h (Oxoid Ltd, Basingstoke, Hampshire, UK). The count of H_2S-producing bacteria (putative *Shewanella* spp.) was carried out on iron agar (Lyngby, Laboratorios Conda, Torrejón de Ardoz, Spain) at 25°C for 48 h. Results were reported as $\log_{10}$ CFU/g meat.

Proximate composition and fatty acid profile analyses

The *Pectoralis major* muscles sampled at 24, 72, and 264 h (corresponding to 1, 7, and 11 d of storage) were individually minced by Grindomix GM 200 (Retsch GmbH, Haan, Germany). An aliquot of fresh minced meat was analysed for fatty acid (**FA**) composition; the remaining meat was freeze-dried, re-ground, and used to determine dry matter (934.01), ash (967.05), crude protein (2001.11), and ether extract (991.36) contents (AOAC, 2000).

Fat was extracted from fresh aliquots by accelerated solvent extraction (ASE®, Dionex, Sunnyvale, CA, USA, Application Note 334) using two extraction cycles with petroleum ether as a solvent at 125°C and 10.3 Mpa, a 6-min heating phase, and a 2-min extraction phase. Then, 10 ml of $NaSO_4$ (0.47% in H_2O) were added to extracted lipids. Samples were kept at 4°C for 30 min and surnatant (petroleum ether and lipids) was collected in another vial previously weighed. Dry evaporation in N_2 stream (Genevac EZ-2, SP Industries, Warminster, PA, USA) was applied; residual samples (extracted lipids in vials) were weighed before adding 2 ml H_2SO_4 2% in methanol (Christie, 1982). Vials were stored at 50°C in heater overnight. Thereafter, lipid rate was calculated; hexane (1 ml hexane/20 mg lipids) and potassium bicarbonate 2% (5 ml) were added. Samples were centrifuged, stored at 4°C for 30 min, and supernatant sampled to be analysed by Agilent 7820A Gas Chromatograph (Agilent Technologies, Santa Clara, CA, USA), with split to 92.199 ml/min and rate set at 65:1. Supelco OMEGAWAX-TM 250 (Sigma-Aldrich,

St. Louis, MO, USA) (30 m × 0.25 mm internal diameter, 0.25 μm film thickness) was used with hydrogen as carrier at 1.4 ml/min. The oven temperature was 50°C, held for 2 min, raised to 220°C at the rate of 4°C/min, held for 17 min. Both the injector and the detector temperatures were set at 250°C.

The FA were identified by comparing the retention time of standard FA methyl esters (**FAMEs**) mixture (Supelco 37 – component FAME Mix, 47885 – U). Individual FAMEs were expressed as the percentage of the total area of eluted FAMEs.

Statistical analysis

The data from technological traits and proximate and FA composition were analysed by ANOVA with meat type (normal, white striping, wooden breast), storage time, and their interaction as the main effects. The PROC GLM of the Statistical Analysis System (SAS, 2013) was used for all analyses. Adjusted means were compared by Bonferroni-t test. Differences between means with $P \leq 0.05$ were accepted as statistically significant differences.

The DMFit program (DMT CO. LTD, Incheon, Korea) modelled the count of each bacteria group to calculate the following microbial growth parameters: initial value ($\log_{10}$ CFU/g), lag phase (h), maximum rate ($\log_{10}$ CFU/g/h), and final value ($\log_{10}$ CFU/g) (Baranyi and Roberts, 1994). The shelf life end was set at 7 $\log_{10}$ CFU/g for TVC and 7.3 $\log_{10}$ CFU/g for *Pseudomonas* (as specific spoilage organisms) (Raab *et al.*, 2008; Rukchon *et al.*, 2014).

The non-parametric combination (**NPC**) test was conducted with the free software NPC Test R10 (http://www.wiley.com/legacy/wileychi/pesarin/material.html). The partial and global *P*-values were calculated for microbial count profiles ($\log_{10}$ CFU/g) considering meat type and storage time. Time was also applied as a stratification block according to the NPC Test's C-sample procedure for dependent variables. Partial *P*-values were corrected for multiplicity and the global *P*-values were obtained using the Tippet combining function.

Hierarchical clustering was applied as an agglomerative approach based on the full linkage method of PRIMER software. Non-metric multidimensional scaling plots visualized samples variability, i.e. dissimilarity between pairs of objects in a two- or three-dimensional space.

RESULTS AND DISCUSSION

Effect of myopathies on meat quality traits

In the present study, normal breasts were the lightest and WB the heaviest (237 g *vs.* 312 g), whereas WS breasts showed intermediate values (280 g) ($P < 0.001$) (Table 5.1). In fact, myopathies occurrence and degrees have been positively associated with breast weight and thickness (Kuttappan *et al.*, 2012a; Mudalal *et al.*, 2015), and with high breast-yield genotypes and older slaughtering age of the birds (Kuttappan *et al.*, 2013 and 2017; Petracci *et al.* 2013b; Lorenzi *et al.*, 2014; Trocino *et al.*, 2015). Overall, under our conditions, the presence of myopathies had weak effects on technological traits. Normal breasts showed lower redness index than WS and WB (−0.88 *vs.* −0.41 and −0.43; $P < 0.001$) and lower cooking losses (22.0% *vs.* 23.8% *vs.* 26.9%; $P < 0.001$) (Table 1), being this last trait also different between WS and WB. Previous studies used breasts within 24-48 h after slaughtering and showed that WS and WB significantly increased meat ultimate pH (Petracci *et al.*, 2013b; Bowker and Zhuang, 2016), especially when both meat abnormalities were simultaneously present (Tasoniero *et al.*, 2016; Zambonelli *et al.*, 2016; Kuttappan *et al.*, 2017). In agreement with the present study, some authors did not report any difference in lightness index as a consequence of myopathies (Petracci *et al.*, 2013b; Trocino *et al.*, 2015; Tasoniero *et al.*, 2016). Differently, other authors found higher lightness in WB than in WS and WS-WB breasts (Mudalal *et al.*, 2015), and in severe-degree WS breasts compared to moderate-degree WS (Sánchez-Brambila *et al.*, 2016).

Literature also reports different results for redness and yellowness indexes. According to some authors, WS (Sánchez-Brambila *et al.*, 2016; Tasoniero *et al.*, 2016) or WB (Zambonelli *et al.*, 2016) did not affect their values. Others observed lower (present study; Petracci *et al.*, 2013b) redness index in normal breasts than in defective ones. Mudalal *et al.* (2015) reported higher yellowness index in WB than in normal meat, with its value increasing with the severity of myopathies and with the simultaneous presence of WS and WB (Kuttappan *et al.*, 2017).

Table 5.1. Effect of myopathy occurrence and storage time on the technological traits of *Pectoralis major* in broiler chickens

	Meat type (M)			Storage time (h) (H)						*P*-value			RMSE[1]
	Normal	White striping	Wooden breast	24	72	120	168	216	264	M	H	M × H	
Breasts (n)	48	48	48	24	24	24	24	24	24				
Weight (g)	237^{c}	280^{b}	312^{a}	277	284	272	272	270	282	<0.001	0.80	0.17	42.4
pH	6.00	6.02	6.05	6.03	6.00	6.03	6.02	6.00	6.05	0.14	0.56	0.80	0.112
L*	46.3	46.4	46.8	48.1^{a}	45.1^{b}	46.6^{ab}	45.9^{b}	47.1^{a}	46.2^{a}	0.29	<0.001	0.54	1.84
a*	−0.88^{b}	−0.41^{a}	−0.43^{a}	-0.64	-0.64	-0.55	-0.57	-0.58	-0.47	<0.001	0.92	0.10	0.594
b*	7.28	7.79	7.77	8.28^{a}	7.64^{ab}	7.41^{ab}	6.98^{b}	7.41^{ab}	7.95^{ab}	0.11	0.02	0.89	1.327
Cooking losses[2] (%)	22.0^{c}	23.8^{b}	26.9^{a}	24.4^{b}	23.9^{b}	22.5^{c}	21.5^{c}	26.9^{a}	26.3^{ab}	<0.001	<0.001	0.05	2.35
Shear force (kg/g)	3.74	3.80	3.39	5.08^{a}	4.15^{b}	2.83^{c}	3.39^{c}	3.50^{c}	2.94^{c}	0.12	<0.001	0.77	1.041

[1] RMSE, root mean square error; SEM is equal to RMSE/√n.

[2] Significant probability of the interaction Meat type × Storage time: Cooking losses, 21.0%, 22.8%, 22.4%, 19.4%, 24.7%, and 23.8% in normal breasts at 24 h, 72 h, 120 h, 168 h, 216 h, and 264 h post mortem; 22.8%, 23.1%, 22.3%, 21.1%, 26.2%, and 27.2% in white striping breasts at 24 h, 72 h, 120 h, 168 h, 216 h, and 264 h post mortem; 29.2%, 25.7%, 24.7%, 24.1%, 29.6%, and 27.8% in wooden breasts at 24 h, 72 h, 120 h, 168 h, 216 h, and 264 h post mortem.

[a,b,c] Means within a row lacking a common superscript differ ($P < 0.05$).

Consistently with the present results, there is a common consent that myopathies increase meat cooking losses especially in the case of WB (Trocino *et al.*, 2015) and with the simultaneous presence of WS and WB (Mudalal *et al.*, 2015; Soglia *et al.*, 2016b; Tasoniero *et al.*, 2016; Zambonelli *et al.*, 2016). Differently, normal and WS breasts may have similar cooking losses (Tasoniero *et al.*, 2016). In fact, a lower ability of holding and binding water has been measured in WB compared to normal and WS meat (Tijare *et al.*, 2016).

Finally, some studies showed that WB breasts had higher shear force values compared to normal and WS breasts (Trocino *et al.*, 2015; Chatterjee *et al.*, 2016; Soglia *et al.*, 2017), but others did not find any significant difference according to myopathy presence (present study; Kuttappan *et al.*, 2013; Mudalal *et al.*, 2015).

The degeneration process in defective muscles at a histological level also affect meat chemical composition (Kuttappan *et al.*, 2012a; Bowker and Zhuang, 2016): myodegeneration is associated to lipidosis (especially in the case of WS) and fibrosis (especially in the case of WB) (Petracci *et al.*, 2014; Soglia *et al.*, 2016b; Radaelli *et al.*, 2017). In fact, in the present trial, WB exhibited the highest water and the lowest protein content (75.0% and 21.4%, respectively) compared to the other breasts (73.4% and 23.9 % in normal, 73.6% and 23.2% in WS, respectively) ($P < 0.001$) (Table 5.2). Moreover, WB meat showed higher ether extract content than normal meat (1.88% *vs.* 1.09%; $P < 0.001$), and WS exhibited intermediate values. Finally, despite the very small range of variation, WB showed the lowest ash content compared to normal and WS samples (Table 2), which may be due to cellular liquid losses and altered ion homeostasis following occurrence of breast myopathies (Petracci *et al.* 2014, Zambonelli *et al.*, 2016).

Table 5.2. Effect of myopathy occurrence and storage time on the proximate composition of *Pectoralis major* in broiler chickens

	Meat type (M)			Storage time (h) (H)			*P*-value			SEM
	Normal	White striping	Wooden breast	24	168	264	M	H	M × H	
Breasts (n)	24	24	24	24	24	24				
Water[1] (%)	73.4[b]	73.6[b]	75.0[a]	74.8[a]	73.8[b]	73.4[b]	<0.001	<0.001	<0.05	0.23
Crude protein (%)	23.9[a]	23.2[a]	21.4[b]	22.2[b]	22.6[b]	23.6[a]	<0.001	<0.001	0.22	0.23
Ether extract (%)	1.09[b]	1.48[ab]	1.88[a]	1.22[b]	1.94[a]	1.29[b]	<0.001	<0.001	0.49	0.128
Ash (%)	1.20[a]	1.20[a]	1.14[b]	1.17[b]	1.16[b]	1.21[a]	<0.001	<0.001	0.18	0.010

[1] Significant probability of the interaction Meat type × Storage time: Water, 74.0%, 73.0%, and 73.2% in normal breasts at 24 h, 168 h, and 264 h post mortem; 73.8%, 73.6%, and 73.3% in white striping breasts at 24 h, 168 h, and 264 h post mortem; 76.7%, 74.7%, and 73.7% in wooden breasts at 24 h, 168 h and 264 h post mortem.
[a,b] Means within a row lacking a common superscript differ ($P < 0.05$).

Other authors also found higher moisture, lipid, and collagen content and lower protein and ash content in WB and WS than in normal meat (Soglia *et al.*, 2016b). Some of these changes increased with the WS degree (Petracci *et al.*, 2014) and when WS and WB were simultaneously present (Soglia *et al.*, 2016a). In fact, the protein functionality also decreases in WS meat, which alters protein intrinsic properties and reduces sarcoplasmic protein solubility (Bowker and Zhuang, 2016). The increased fat/protein and collagen/protein ratio of affected meat finally implies a decreased nutritional quality (Petracci *et al.*, 2014).

In our trial, a significant interaction was found between meat quality and storage time ($P < 0.05$): water content was higher in WB (76.7%) than in N and WS meat (74.0% and 73.8%, respectively) only 24 h after slaughtering, whereas no differences among groups were found at 168 h or 264 h post mortem (Table 2). Likely, protein denaturation during storage altered the water holding capacity of meat (Hughes *et al.*, 2014; Warner, 2017), which in turn increased water losses and reduced water content of all samples, as described during chilling and frozen storage in lamb meat and pork (Coombs *et al.*, 2017; Medić *et al.*, 2018).

Fatty acid profile was similar in WS and WB meat. Differently, compared to normal breasts, WB and WS samples exhibited lower saturated FA (**SFA**) (31.3% *vs.* 28.0% and 28.0%) and higher unsaturated FA (**UFA**) (68.7% *vs.* 72.0% and 72.0%) and, thus, a lower SFA/UFA ratio (0.46 *vs.* 0.39 and 0.39) ($P < 0.001$) (Table 3). Differences found in UFA were mainly due to variations in polyunsaturated FA (PUFA), which were higher in affected than in normal breasts (35.3% in WS and 35.4% in WB *vs.* 30.5% in normal, respectively; $P < 0.001$). Conversely, monounsaturated FA (**MUFA**) were lower in WS and WB (36.7%) than in normal meat (38.2%) ($P < 0.05$). Furthermore, both n-3 and n-6 FA proportions were higher in WB and WS than in normal samples ($P < 0.001$).

Among individual FA, palmitoleic (C16:1 n7) and oleic (C18:1 n9) acids primarily accounted for differences in MUFA between affected and normal meat ($P < 0.05$); among PUFA, linoleic (C18:2 n6) and α-linolenic (C18:3 n3) acids mostly changed (Table 5.3).

Table 5.3. Effect of myopathy occurrence and storage time on the FA composition (% total FA) of *Pectoralis major* in broiler chickens

	Meat type (M)			Storage time (h) (H)			*P*-value			
	Normal	White striping	Wooden breast	24	168	264	M	H	M × H	SEM
Breasts (n)	24	24	24	24	24	24				
C14:0	0.45	0.43	0.43	0.43^{ab}	0.42^{b}	0.45^{a}	0.41	<0.05	0.43	0.008
C16:01	22.8^{a}	20.4^{b}	20.4^{b}	20.9	20.9	21.7	<0.001	0.10	<0.05	0.31
C16:1 n9	0.38^{b}	0.42^{a}	0.43^{a}	0.43^{a}	0.40^{b}	0.40^{b}	<0.001	<0.05	0.82	0.006
C16:1 n7	3.58^{a}	3.19^{b}	3.14^{b}	3.32	3.52	3.08	<0.05	0.06	0.60	0.128
C18:0	7.48^{a}	6.63^{b}	6.59^{b}	6.72^{b}	6.38^{b}	7.60^{a}	<0.001	<0.001	0.35	0.126
C18:1 n9	31.8^{a}	30.7^{b}	30.8^{b}	30.8	31.7	30.8	<0.05	0.11	0.10	0.33
C18:1 n7c	1.78	1.73	1.71	1.73^{ab}	1.69^{b}	1.80^{a}	0.13	<0.05	0.34	0.020
C18:2 n62	25.6^{b}	29.9^{a}	29.7^{a}	29.0	28.8	27.4	<0.001	0.12	<0.05	0.57
C18:3 n3	2.58^{b}	3.06^{a}	3.08^{a}	2.97^{ab}	3.00^{a}	2.75^{b}	<0.001	<0.05	0.15	0.061
C20:4 n6	0.69	0.72	0.79	0.77^{ab}	0.61^{b}	0.83^{a}	0.34	<0.001	0.27	0.061
C20:5 n3	0.07	0.06	0.08	0.07	0.07	0.08	0.27	0.20	0.99	0.005
C22:6 n3	0.07	0.07	0.08	0.08	0.07	0.08	0.21	0.89	0.84	0.006
SFA3	31.3^{a}	28.0^{b}	28.0^{b}	28.6^{b}	28.2^{b}	30.4^{a}	<0.001	<0.001	<0.05	0.37
UFA4	68.7^{b}	72.0^{a}	72.0^{a}	71.4^{a}	71.8^{a}	69.6^{b}	<0.001	<0.001	<0.05	0.37
MUFA	38.2^{a}	36.7^{b}	36.7^{b}	36.9	37.8	36.8	<0.05	0.17	0.14	0.43
PUFA5	30.5^{b}	35.3^{a}	35.4^{a}	34.4	34.0	32.8	<0.001	0.26	<0.05	0.69
∑ n-3	2.89^{b}	3.34^{a}	3.39^{a}	3.26	3.28	3.08	<0.001	0.14	0.23	0.078
∑ n-6	27.4^{b}	31.7^{a}	31.7^{a}	30.9	30.4	29.5	<0.001	0.28	<0.05	0.62

SFA, saturated FA; UFA, unsaturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated FA.

[1] Significant probability of the interaction Meat type × Storage time: C16:0, 23.3%, 21.9%, and 23.1% in normal breasts at 24 h, 168 h, and 264 h post mortem; 19.5%, 19.9%, and 21.7% in white striping breasts at 24 h, 168 h, and 264 h post mortem; 19.9%, 20.8%, and 20.3% in wooden breasts at 24 h, 168 h, and 264 h post mortem.

[2] Significant probability of the interaction Meat type × Storage time: C18:2 n6, 25.0%, 27.0%, and 24.9% in normal breasts at 24 h, 168 h, and 264 h post mortem; 31.6%, 30.8%, and 27.2% in white striping breasts at 24 h, 168 h, and 264 h post mortem; 30.5%, 28.5%, and 30.2% in wooden breasts at 24 h, 168 h and 264 h post mortem

[3] Significant probability of the interaction Meat type × Storage time: SFA, 31.8%, 29.6%, and 32.4% in normal breasts at 24 h, 168 h, and 264 h post mortem; 26.7%, 27.1%, and 30.2% in white striping breasts at 24 h, 168 h and 264 h post mortem; 27.4%, 27.9%, and 28.5% in wooden breasts at 24 h, 168 h, and 264 h post mortem.

[4] Significant probability of the interaction Meat type × Storage time: UFA, 68.2%, 70.4%, and 67.6% in normal breasts at 24 h, 168 h, and 264 h post mortem; 73.3%, 72.9%, and 69.8% in white striping breasts at 24 h, 168 h, and 264 h post mortem; 72.6%, 72.0%, and 71.5% in wooden breasts at 24 h, 168 h, and 264 h post mortem.

[5] Significant probability of the interaction Meat type × Storage time: PUFA, 29.6%, 31.9%, and 30.0% in normal breasts at 24 h, 168 h and 264 h post mortem; 37.2%, 36.3%, and 32.5% in white striping breasts at 24 h, 168 h, and 264 h post mortem; 36.4%, 33.7%, and 36.1% in wooden breasts at 24 h, 168 h, and 264 h post mortem.

[6] Significant probability of the interaction Meat type × Storage time: ∑ n6, 26.5%, 28.6%, and 27.0% in normal breasts at 24 h, 168 h, and 264 h post mortem; 33.4%, 32.5%, and 29.1% in white striping breasts at 24 h, 168 h, and 264 h post mortem; 32.7%, 30.1%, and 32.3% in wooden breasts at 24 h, 168 h, and 264 h post mortem.

[a,b] Means within a row lacking a common superscript differ ($P < 0.05$).

Poultry meat has a high nutritional value due to its high protein and low lipid contents as well as its high PUFA rate. As measured in our data, linoleic acid (C18:2 n6) and α-linolenic acid (C18:3 n3) contribute to the high UFA rate (Grashorn, 2017). Indeed, FA composition of the meat is notoriously influenced by the lipid dietary intake (Liburn, 2017), but few data are available on the effect of myopathies on FA meat composition. Some authors found higher SFA rates in normal breasts than in WS and WB ones (Kuttappan *et al.*, 2012a; Soglia *et al.*, 2016a), consistently with the results of the present trial, whereas others (Traffano-Schiffo *et al.*, 2017) observed an opposite trend. Soglia *et al.* (2016a) did not find any significant difference in total MUFA or PUFA. Nevertheless, they found that linoleic acid (C18:2 n6) rate was lower in normal compared to WB and WS/WB breasts, and α-linolenic acid (C18:3 n3) rate was lower in normal breasts compared to WS/WB breasts. These last findings are consistent with the results of Kuttappan *et al.* (2012a) and those of the present study.

The comparison with the results of Traffano-Schiffo *et al.* (2017) is difficult because they measured FA composition by Differential Scanning Calorimetry on the superficial meat layers. They found that WS samples had approximately 5% SFA, 23% PUFA, and 72% MUFA, whereas normal breasts had no SFA, 76% PUFA, and 24% MUFA.

The increased PUFA content in WS and WB meat reported by several authors on the whole muscle could be associated to sarcolemma damages, inflammatory processes, and consequent myopathy development (Kuttappan *et al.*, 2012a; Soglia *et al.*, 2016a). On the other hand, the increased SFA content in the superficial layer of breasts affected by WS (Traffano-Schiffo *et al.*, 2017) could be explained by the lipidosis that can be observed at this specific level. Further research is necessary to clarify the mechanism and the causes of changes in FA profile of poultry meat affected by myodegeneration.

Effect of storage time on meat quality traits

The breast weight of the different batches under analyses during storage was the same (276 g on average; Table 1). Under our conditions, meat pH remained stable until the end of storage (11 d). In fact, a longer time (15 d) would be necessary to record increased pH as a consequence of the accumulation of protein degradation products (Marcinkowska-Lesiak *et al.*, 2016).

The L* index decreased from 24 h (48.1) to the following sampling hours (45.9 on average), but it significantly increased again (47.1) at 216 h ($P < 0.001$) (Table 1). The b* index showed the highest value after 24 h and the lowest value after 168 h (8.28 *vs.* 6.98; $P < 0.05$), without significant differences with and among the other sampling times. Cooking losses during the first 72 h post mortem averaged at 24.2%, decreased until 22.0% between 120 h and 168 h, and finally reached on average 26.6% between 216 h and 264 h ($P < 0.001$). Besides, the differences among storage times were different according to meat type (probability of the interaction, $P = 0.05$) (Table 1). Cooking losses of WB breasts significantly changed from 29.2% on the first sampling time (24 h) to lower values between 72 h and 168 h (24.8% on average), and

finally increased again at 216 h and 264 h (29.6% and 27.8%, respectively). Lastly, the highest shear force was measured at 24 h of storage time (5.08 kg/g) to decrease by 72 h and to reach the lowest value by 120 h (2.83 kg/g; $P < 0.001$). From this time onwards, shear force remained stable (Table 1).

Changes in lightness, cooking losses, and shear force during storage were consistent with changes in water retention capability and myofibrils structure of the meat. The high initial meat lightness and cooking losses likely depend on the high rate of free water in meat; later during storage, meat lost free water by dripping; finally, lightness and cooking losses increased again likely because the water previously linked to myofibrils was released with protein denaturation and degeneration. Myofibrils changes also explains the reduction of meat shear force during storage as observed by previous studies (Soglia *et al.*, 2017; Sun et al, 2018) both in normal and WB meat. In line with our results, Sun *et al.* (2018) also found a similar reduction of the compression force over a 8-d storage time at 4°C in all breast categories (normal, mild and moderate/severe WB), and higher changes in drip loss in moderate/severe WB than in normal breasts over time.

Meat water content was higher at 24 h and significantly decreased by 168 h and 264 h post mortem (74.8% *vs.*73.8% and 73.4%; $P < 0.001$) (Table 2). On the contrary, crude protein content was lower at 24 h and 168 h and increased by 264 h (22.2% and 22.6% *vs.* 23.6%; $P < 0.001$). Similar changes were observed for ash content ($P < 0.001$). Variations in ether extract content, despite significant ($P < 0.001$), were in a narrow range. Likely, changes in water content depended on the higher drip losses of aged meat, which in turn may have increased the relative proportion of crude protein in meat after 264 h, consistently with previous results (Coombs *et al.*, 2017; Medić *et al.*, 2018).

As time passed, chicken breasts also changed their FA profile (Table 3). In fact, SFA rate increased (28.6% and 28.2% at 24 h and 168 h *vs.* 30.4% at 264 h; $P < 0.001$) due to increased rates of C16:0 (20.9% to 21.7%; $P = 0.10$) and C18:0 from 24 h and 168 h to 264 h (6.72% and 6.38% *vs.* 7.60%; $P < 0.001$). The most abundant UFAs were oleic acid (C18:1 n9) and linoleic acid (C18:2 n6), which rates did not change over time. Nevertheless, small changes in other MUFA and PUFA accounted for a reduction in UFA rates from 24 h and 168 h to 264 h (71.4% and 71.8% *vs.* 69.6%; $P < 0.001$). Some differences were recorded across meat type (probability of the interaction meat type × storage time, $P < 0.05$). Normal breasts had lower SFA and C16:0 rates and higher UFA rates at 168 h than at 24 or 264 h; WS showed lower C18:2 n6 rate and, consequently, lower PUFA and n-6 rates at the end of storage (264 h). These changes were not observed in the other meat types.

The increase of SFA rate with storage time may be due to changes in MUFA and, especially, PUFA contents, which are expected to decrease during storage because of lipid oxidation (Wood, 2017; Medić *et al.*, 2018)

Microbiological shelf life of meat

Under optimal chilling conditions, fresh poultry meat can have 4-10 d of shelf life depending on the packaging solution (Patsias *et al.*, 2008; Raab *et al.*, 2008; Vasconcelos *et al.*, 2014). Nevertheless, among food, poultry meat has the highest pathogenic and spoilage bacteria counts (Galarz *et al.*, 2016) and the liable organisms for spoilage are *Pseudomonas* spp., *Shewanella putrefaciens, Acinetobacter, Moraxella, Psychrobacter* spp., including *A. johnsonii* and *P. immobilis*, and *Enterobacteriaceae* spp. (Mead, 2004; Rouger *et al.*, 2017).

Several indicators are proposed to evaluate the shelf life of poultry meat, among them TVC and *Pseudomonas* ssp. are the most applied descriptors (Rouger *et al.*, 2017; Bruckner *et al.*, 2012). In the present trial, microbial growth parameters of TVC and *Pseudomonas* ssp. were different between normal and affected meat during storage. In fact, normal breasts had the highest initial TVC value and the shortest TVC lag phase in comparison to WS and WB breasts (Table 4). Differences in lag phases indicate that WS and WB samples undergone a later microbial spoilage, their exponential growth started after 85.2 h and 77.8 h of storage time, respectively, than normal ones (46.3 h). Therefore, shelf life threshold (7 $\log_{10}$ CFU TVC/g) was achieved sooner in normal breasts (130 h) than in WS (149 h) and WB (192 h). After this threshold was reached, TVC final count was similar in normal and WB meat, but lower in WS samples (Table 5.4).

Table 5.4. Estimated growth parameters (± SE) of total viable counts and *Pseudomonas* spp. of *Pectoralis major* in normal, white striping and wooden breasts of broiler chickens

	Normal	White striping	Wooden breast
Breasts (n)	30	30	30
Total viable counts (TVC)			
Initial value ($\log_{10}$ CFU/g)	3.70 ± 0.17	3.39 ± 0.17	3.20 ± 0.15
Lag phase (h)	46.3 ± 9.36	85.2 ± 9.44	77.8 ± 8.80
Maximum rate ($\log_{10}$ CFU/g/h)	0.040 ± 0.004	0.062 ± 0.001	0.035 ± 0.003
Final value ($\log_{10}$ CFU/g)	8.24 ± 0.13	7.53 ± 0.17	8.21 ± 0.26
Shelf life (h (d))	130 (5)	149 (6)	192 (8)
R^2	0.96	0.93	0.96
SE fit	0.39	0.54	0.37
Pseudomonas spp.			
Initial value ($\log_{10}$ CFU/g)	3.00 ± 0.30	2.43 ± 0.23	3.69 ± 0.19
Lag phase (h)	27.4 ± 13.3	60.9 ± 12.4	77.4 ± 6.9
Maximum rate ($\log_{10}$ CFU/g/h)	0.046 ± 0.008	0.067 ± 0.015	0.066 ± 0.009
Final value ($\log_{10}$ CFU/g)	8.25 ± 0.2	7.72 ± 0.19	7.59 ± 0.18
Shelf life (h (d))	122 (5)	139 (6)	154 (6)
R^2	0.92	0.94	0.94
SE fit	0.64	0.61	0.55

Pseudomonas ssp. are commonly considered a specific spoilage organism for several types of food, mainly pork, poultry, and seafood. This microbial target is directly involved in spoilage mechanisms due to its ability to produce pigmented molecules, extracellular enzymes, slimes, and off-odors (Andreani and

Fasolato, 2017). Estimated *Pseudomonas* ssp. growth was somewhat different from TVC evolution in the present trial (Table 4). *Pseudomonas* ssp. count was initially lower in normal and WS meat than in WB meat; during storage, normal breasts showed a shorter lag phase (27.4 h *vs.* 60.9 h and 77.4 h) and a lower *Pseudomonas* growth rate compared to abnormal ones. Then, normal samples exhibited a higher final *Pseudomonas* spp. count than WS and WB meat. The spoilage threshold proposed by Raab *et al.* (2008) for *Pseudomonas* spp. (7.3 $\log_{10}$ CFu/g) was achieved first in normal breasts compared to WS and WB (122 h *vs.* 139 h and 154 h). The estimated shelf life obtained in this work for normal and abnormal breasts is consistent with previous data for poultry meat stored under different conditions (chilling at 4°C, freeze chilling, packaging with or without modified atmosphere) (Raab *et al.*, 2008; Patsias *et al.*, 2008).

The global *P*-value of the full model, which tested the effects of meat type and storage time, showed an overall effect on the different microbial target counts ($P < 0.001$) (Table 5). About the single effects, meat type was significant only for *Enterobacteriaceae* spp. count, whereas storage time was significant for all targets. Nevertheless, the NPC tests among meat types stratified by storage time showed that TVC and *Pseudomonas* spp. counts significantly changed with meat type between 72 h and 216 h of storage (Table 5). During this period, normal meat showed the highest microbial counts compared to breasts affected by myopathies. At intermediate sampling times (120 h and 168 h of storage), the difference between normal and abnormal meat reached about +2 $\log_{10}$ CFU/g. Moreover, NPC tests showed that *Enterobacteriaceae* spp. and LAB counts significantly changed with meat type since the first sampling, after 24 h of storage, and until 216 h. Overall, normal meat displayed the highest load, WB the lowest one, whereas WS had intermediate values. All differences in microbial targets across meat types disappeared by 264 h of storage (i.e. 11 d). Literature reports *Pseudomonas* spp. as dominant in poultry meat stored under aerobic conditions (Vasconcelos *et al.*, 2014; Rouger *et al.*, 2017), but in our trial most of microbial targets reached 7 $\log_{10}$ CFU/g by the storage end (Table 5.5).

Table 5.5 Effect of myopathy occurrence and storage time on microbial targets (mean ± SD) (log_{10} CFU/g) of *Pectoralis major* in broiler chickens (global and partial *P*-values are referred to the non-parametric combination test)

Storage time (h)	Meat type	TVC[1]	*Pseudomonas* spp.	H_2S producer	*Entero-bacteriaceae*	LAB[2]	Global *P*-value
Tests among meat types stratified by storage time							<0.001
24	Normal	3.72 ± 0.30	3.01 ± 0.58	2.58 ± 0.14	2.45 ± 0.32	3.22 ± 0.44	
	White striping	3.67 ± 0.34	2.36 ± 0.32	2.44 ± 0.18	2.32 ± 0.30	3.63 ± 0.29	
	Wooden breast	3.34 ± 0.09	2.68 ± 0.57	2.12 ± 0.72	1.84 ± 0.29	2.85 ± 0.27	
	P-value	0.08	0.17	0.15	<0.001	<0.01	
72	Normal	4.04 ± 0.31	4.16 ± 1.09	2.90 ± 0.36	2.96 ± 0.06	3.61 ± 0.16	
	White striping	3.10 ± 0.16	2.71 ± 0.56	2.60 ± 0.42	2.19 ± 0.22	3.47 ± 0.22	
	Wooden breast	3.21 ± 0.14	2.70 ± 0.51	2.59 ± 0.35	2.17 ± 0.42	3.22 ± 0.40	
	P-value	<0.001	<0.001	0.37	<0.001	0.13	
120	Normal	5.85 ± 0.28	6.08 ± 0.78	3.69 ± 0.17	4.26 ± 0.45	4.74 ± 0.37	
	White striping	4.26 ± 0.13	4.71 ± 0.28	3.78 ± 0.46	3.44 ± 0.43	3.95 ± 0.11	
	Wooden breast	3.90 ± 0.31	4.02 ± 0.45	3.48 ± 0.47	3.01 ± 0.24	3.43 ± 0.55	
	P-value	<0.001	<0.001	0.50	<0.001	<0.001	
168	Normal	7.51 ± 0.48	8.07 ± 0.51	5.62 ± 0.70	6.32 ± 0.22	5.69 ± 0.60	
	White striping	6.92 ± 0.51	7.51 ± 0.51	5.99 ± 0.94	4.93 ± 0.55	5.11 ± 0.43	
	Wooden breast	5.83 ± 0.25	6.91 ± 0.48	5.44 ± 0.46	4.56 ± 0.66	4.48 ± 0.79	
	P-value	<0.001	<0.01	0.51	<0.001	<0.05	
216	Normal	8.10 ± 0.64	8.03 ± 0.24	6.22 ± 0.44	6.40 ± 1.02	6.52 ± 0.36	
	White striping	7.07 ± 0.51	7.11 ± 0.58	5.23 ± 0.98	5.23 ± 1.00	5.48 ± 0.95	
	Wooden breast	6.90 ± 0.48	7.01 ± 0.57	5.48 ± 0.27	4.11 ± 1.00	5.40 ± 0.56	
	P-value	<0.05	<0.01	0.06	<0.01	<0.05	
264	Normal	8.11 ± 0.59	8.31 ± 0.41	6.83 ± 0.28	7.48 ± 0.46	6.97 ± 0.80	
	White striping	7.98 ± 0.59	8.29 ± 0.51	6.82 ± 0.21	7.09 ± 0.60	7.07 ± 0.39	
	Wooden breast	8.09 ± 0.54	8.03 ± 0.47	6.77 ± 0.20	6.71 ± 0.58	6.80 ± 0.43	
	P-value	0.93	0.57	0.92	0.13	0.77	
Tests among levels of meat type and storage time factors							
Meat type	Partial *P*-value	0.15	0.65	0.75	<0.01	0.16	<0.05
Storage time	Partial *P*-value	<0.01	<0.001	<0.01	<0.001	<0.001	<0.001

[1] TVC, total viable counts; [2] Lactic acid bacteria.

Lastly, the non-metric multidimensional scaling plot in Figure 5.1, based on all microbial targets, shows two main cluster groups. The first cluster includes all samples at 24 h and 72 h of storage associated with WS and WB samples at 120 h (20% similarity). The second cluster includes all other samples (20% similarity). Within the first cluster, samples at 24 h are separated from samples at 120 h (80% similarity). Within the

second cluster, normal breasts at 120 h are grouped with WS and WB samples analysed at 168 h and 216 h (60% similarity); and normal breasts at 216 h are grouped to samples analysed at 264 h (80% similarity).

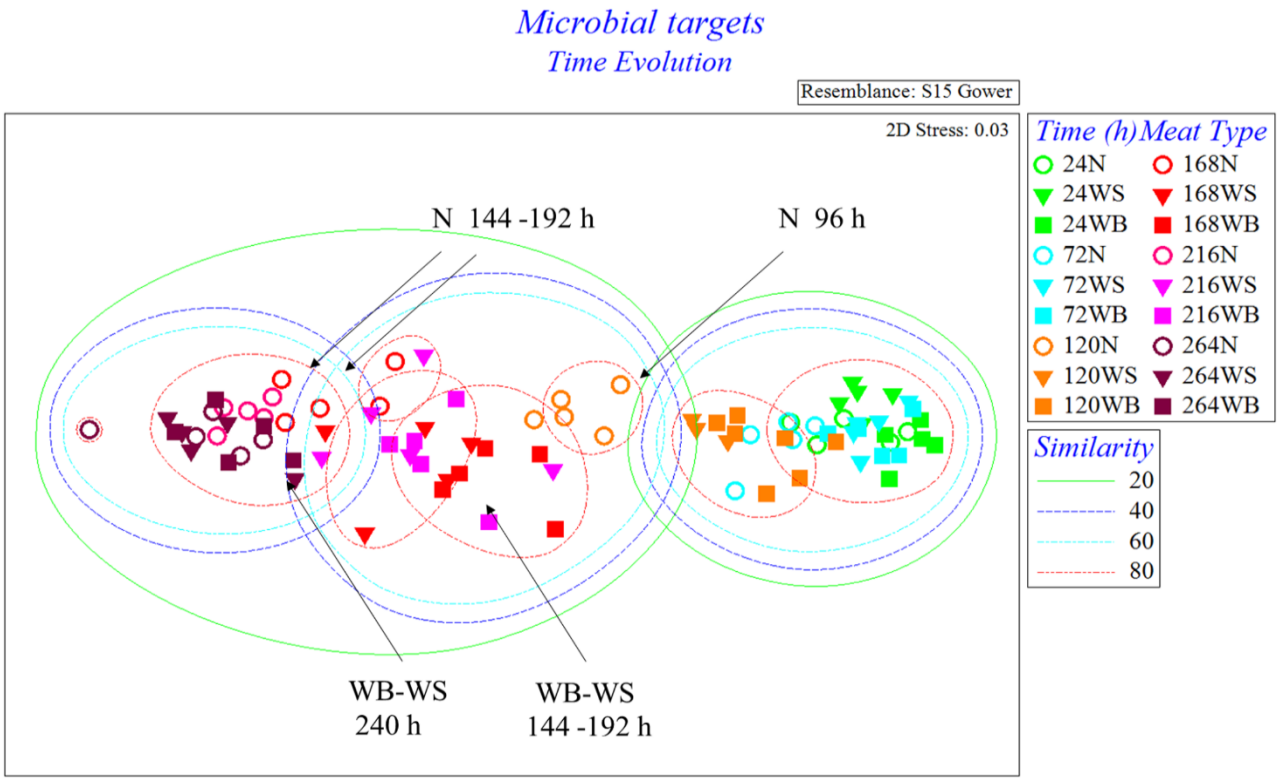

Figure 5.1. Non-metric multidimensional scaling plot overlapped with the similarities obtained by hierarchical cluster analysis of samples. Data are labelled according to the storage time (different colors); Circles = N, normal breasts; Triangles = WS, white striping breasts; Square = WB, wooden breasts. Some peculiar cluster was highlighted with arrows (h, h post mortem).

The microbiological profile of poultry meat affected by myopathies has been little investigated. The only study available to our knowledge (Dalgaard *et al.*, 2018) used storage conditions different from ours. In fact, these authors evaluated microbiological profile of normal and moderate or severe WB breasts after 6 d and 8 d of storage at 4°C in modified atmosphere packaging (70% nitrogen, 30% CO_2). Under these conditions, at the end of the storage, TVC on meat surface did not differ between normal and WB meat. Nevertheless, *Enterobacteriaceae* spp. counts in severe WB samples significantly increased from 6 d to 8 d of storage. According to the same authors, alterations in muscle tissue, moisture content, mobile water fraction, drip loss, and pH affected the microflora growth and composition in WB samples stored in modified atmosphere packaging, favouring *Enterobacteriaceae* spp. and microflora diversification. In fact, water holding capacity, protein, and collagen quality of the meat are known to affect microbial proliferation (Maxcy, 1981). The growth of some foodborne bacteria (specifically *E. coli* O157:H7) is dependent on their ability of adhering to muscle fiber extracellular matrix (endomysium, perimysium, epimysium) (Chagnot *et al.*, 2017). Thus, the alteration in muscle tissues of WS and WB meat may modify muscle environment and, consequently, microflora growth and composition (Dalgaard *et al.*, 2018). In our study, also differences in FA composition

between normal and affected meat could have played a role. In fact, antimicrobial activities of long-chain UFA compared to long-chain SFA are documented (Zheng *et al.*, 2005). These FAs have inhibitory effects both on Gram (+) and Gram (−) bacteria (Lee *et al.*, 2002), likely due to their action at bacterial cell membranes, and changes in FA composition are under study as a strategy to slow down or prevent food spoilage (Lee *et al.*, 2002; Zheng *et al.*, 2005; Desbois and Smith, 2010).

In conclusion, the present study confirmed that myopathy presence brings about changes in meat technological traits, and, especially, proximate and fatty acid composition. This fact challenges nutritive value, besides processing ability of the meat. Overall quality of normal and abnormal meat have a similar evolution during storage at 4°C under aerobic conditions, but surprisingly microbial shelf life is shorter in normal than in abnormal meat. Further studies are necessary to elucidate the factors and the mechanisms that may modulate microbial growth and composition during storage in poultry meat affected by myopathies.

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GENERAL DISCUSSION

The present PhD thesis aimed at evaluating and defining different strategies to control the occurrence of myopathies and muscle fiber degeneration in broiler chickens as well as at evaluating quality of abnormal meat. Four different trials were carried out to achieve this objective.

The **first contribution** compared two genetic lines, of both genders, submitted to two different feeding strategies (*ad libitum* and a quantitative feed restriction, 80% respect to *ad libitum* diet) in the first growing period (13 to 21 d of age). Moreover, muscle fiber histological and immune-histochemical changes were evaluated at different ages (14, 21, 28, 35, and 46 d) associated with the occurrence of myopathies in the pectoralis major muscle fibers.

The **second contribution** aimed at deepening the investigation about the effect of feeding strategies, *ad libitum* diet, early (from 13 to 23 d of age) and late (from 27 to 37 d of age) feed restriction in male broiler chickens belonging to two genotypes (Cobb 500 and Ross 308). Also in this case, interactions between feeding regime and age of animals on muscle fiber degeneration were tested.

The **third contribution** investigated the effect of a management strategy, like the modulation of photoperiod, with the objective of reducing the growth rate. Thus, this contribution studied how two different daylights (14 hours of light *vs.* 18 hours of light) might affect productive performance, meat quality and traits, and occurrence and severity of myopathies belonging to two commercial genotypes and both genders.

Finally, the **fourth contribution** aimed at comparing technological, chemical and microbiological traits in normal, white striped, and woody breast. Moreover, the rheological and microbial shelf-life was tested during a storage of 11 days.

Effect of feed restriction

Whereas the aetiology of myopathies is not yet clear, numerous factors, which can modulate the occurrence and the severity of myopathies, have been investigated and their influence reported also by other Authors. The first Trial evaluated the possibility of decreasing the occurrence and severity of alterations limiting the growth rate by feed restriction. This technique achieved the goal only partially; indeed, animals fed under a regime of feed restriction showed a decrease in occurrence of myofiber degeneration only when submitted to treatment, but when they returned to ad libitum feeding the positive result was lost. Similarly, in the second trial, feed restriction, either early or late, did not affect either the occurrence or severity of the myopathies, even if the re-alimentation period permitted animals to perform compensatory growth especially in the case of early restriction. On the other hand, in the third trial, the use of two different photoperiods, which resulted in a feeding restriction due to behaviour of broiler chicken, significantly affected occurrence and, especially,

severity of white striping. In the third trial, differences in light management were maintained until the end of the trial so that no compensatory growth was observed.

Generally speaking, and as expected, the feeding regime and the lighting regime that affected feed intake conditioned (and impaired) growth rate of chickens during the restriction period, as previously found by several authors adopting different feed restriction plans and systems (Urdaneta-Rincon and Leeson, 2002; Zhan *et al.*, 2007; Butzen *et al.*, 2013). During the re-alimentation, however, previously restricted birds showed a compensatory growth, which allowed birds to reduce differences in final live weight and improved feed conversion rate. However, the duration of the re-alimentation period in the first trial and the timing of re-alimentation in the case of late-restricted chickens of the second trial were not sufficient to permit the full recovery of live weight, and some residual effects were still evident in terms of reduced performance as well as dressing percentage, breast and thighs yield in the restricted birds as reported by others (Urdaneta-Rincon and Leeson, 2002; Zhan *et al.*, 2007; Butzen *et al.*, 2013). As reviewed by Sahraei (2012), in fact, the duration, timing, and severity of feed restriction as well as the re-alimentation terms affect the occurrence and degree of the compensatory growth and, therefore, the residual effects on carcass quality.

Controlling the occurrence and the degree of myopathies would be of great interest for the poultry industry in view of preventing and containing negative effects on consumers acceptance (Kuttappan *et al.*, 2012b) and meat technological properties (Mudalal *et al.*, 2014; Mazzoni *et al.*, 2015). Indeed, myopathies and associated MFD degeneration occur early and increase with age (14 d of age in trial 1 of the present thesis; 16 d in Griffin *et al.*, 2018; 18 d in Sihvo *et al.*, 2017) and timing of feeding and management strategies must consider this data.

The correlation between white striping occurrence and growth rate/final live weight and/or breast yield in current broiler genotypes under commercial intensive conditions has been stated in all trials of the present thesis. High growth rates and high breast yield have also been considered responsible for wooden breasts also by other authors (Sihvo *et al.*, 2014). Indeed, according to Bailey *et al.* (2015), environmental and/or management factors may contribute to more than 65% and 90% of the variance in the occurrence of white striping and wooden breast, respectively. In fact, these authors found that heritability was low both for wooden breast (<0.10) and white striping (<0.34) and genetic correlations between breast myopathies and body weight ranged from less than 0.132 to 0.248. On the other hand, recently, a strong genetic determinism of the WS condition in fast-growing chickens was found ($h^2 = 0.65$; Alnahhas *et al.*, 2016).

In our studies, feed restriction was used to control bird growth and thus muscle fibre accretion. Accordingly, during restriction both in trial 1 and trial 2 of the present thesis, feed restriction successfully reduced the occurrence of muscle fibre degeneration compared to ad libitum feeding. During the subsequent re-feeding period, however, restricted birds showed a compensatory growth, which induced also an important damage at fibre level. In fact, myopathies have been associated with an increased muscle hypertrophy of fast-growing chickens which brings about reduced capillary density adjacent to the myofiber (Hoving-Bolink *et al.*, 2000;

Joiner *et al.*, 2014) thus affecting regeneration, degeneration and necrosis of skeletal muscles (Velleman, 2015). Based on gene expression and pathway by RNA-sequencing, localized hypoxia, oxidative stress, increased intracellular calcium, and the presence of muscle fiber-type switching have been proposed as responsible for wooden breast and white striping (Mutryn *et al.*, 2015; Soglia *et al.*, 2016b; Marchesi *et al.*, 2018). Indeed, Kuttapan *et al.* (2012) reduced the occurrence of white striping in broilers slaughtered at 54 d of age by lowering the energy value of diets (75% to 53%; $P < 0.05$). Consistently, Livingston *et al.* (2018) reduced white striping (90% to 41%) and wooden breast (95% to 86%) rate as well as their degree (2.87 to 1.64 for white striping and 2.89 to 2.14 for wooden breast) in male chickens at 42 d of age. On the other hand, Meloche *et al.* (2018a) successfully reduced WS and WB severity at different ages (33, 43, and 50 d) according to feed restriction rate (from 95% to 85% of ad libitum). However, the myopathies reduction was obtained at the expenses of performance and carcass traits (Kuttappan *et al.*, 2012; Livingston *et al.*, 2018). On the other hand, when early feed restriction was used to prevent myopathies and safeguard productive performance, the occurrence of white striped breasts at commercial slaughter (46 d) tended to increase (Trocino *et al.*, 2015), whereas at histological examination 97% of breasts showed MFD without significant differences between early restriction and ad libitum feeding (First contribution). These results were ascribed to the fast (muscle) growth rate of early-restricted chickens related to their compensatory growth during the refeeding period compared with birds always fed ad libitum.

In fact, white striping occurrence in broilers slaughtered at 54 d of age was reduced only when growth rate was controlled during the whole rearing period by the administration of low energy diets (Kuttappan *et al.*, 2012) or reducing diet intake by reducing the number of light hours (Third contribution). On the other hand, a too early feed restriction (first two weeks post hatching) had negative effects on P. major structure, in terms of poor organization, increased necrosis, and fat deposition, which were still fully evident at 42 d of age (Velleman *et al.*, 2010).

Effect of gender

Within the present thesis, the effect of gender was always significant on performance, slaughter results, and myopathy occurrence when tested (First and Third contribution). Males and females showed large differences in productive performance and meat quality. Male chickens reached higher live weight whereas they showed higher dressing out and less breast yield compared to females. Moreover, meat quality was affect by gender even if not always consistently along the two trials. Meat from females showed lower pH, higher cooking losses, and higher shear force than males. Lower pH of P. major in females than in males have been previously reported also by other authors (López *et al.*, 2011; Brewer *et al.*, 2012; Schneider *et al.*, 2012), but lightness and red indexes and water holding capacity were similar; the higher yellow index measured in females than in males has been reported also by Schneider *et al.* (2012).

Nevertheless, white striping rate and degree did not change in the two genders, whereas the occurrence of wooden breast was largely associated to the male chickens compared to females. In fact, high growth rates and high breast yield have also been considered responsible for wooden breasts (Sihvo *et al.*, 2014), but the only significant difference in wooden breast occurrence has been reported between heavy males and light females slaughtered at the same age (Trocino *et al.*, 2015). Nevertheless, no difference between genders was recorded in muscle fiber degeneration score. On the other hand, recent records showed that females show higher rates of another emergent myopathy, i.e. spaghetti meat, compared to males. According to Trocino *et al.* (2018), gender does not affect WS, whereas WB rates were lower (7.28% *vs.* 21.9%; $P<0.01$) and SM rates higher (25.2% *vs.* 3.13%; $P<0.001$) in females than males.

Effect of genotype

The genotypes tested in the trials appeared different on productive performance and meat quality; indeed, if in the first trial we compared standard breast yield and high breast yield in second and third we compared two different genetic hybrids selected for high breast yield: Ross 308 and Ross 708. In the first experiment (First contribution) we observed a significant difference in live weight, with higher values for standard breast yield and lower values in dressing out for standard breast yield genotype. Less effects were measured on meat quality. Indeed, in the second Trial (Second contribution) we tested two commercial genotypes, Cobb vs, Ross, and we found differences in growth and slaughter results that were not consistent between trials. In fact, comparison between broiler genotypes within the same trial, from one trial to another and with literature data is difficult because results may be affected by several factors, like hatchery, reproductive stock age and condition, genotype nutritional requirements, and feeding plans. In fact, in the second contribution, Cobb showed lower performance and carcass traits, whereas in the third contribution an opposite trend was observed. Nevertheless, key information may be obtained about prevalence of myopathies and interactions with growth rates (Kuttappan *et al.*, 2012; Petracci *et al.*, 2013a; Trocino *et al.*, 2015). Some authors outlined differences between genotypes along the growth period: high-breast yield genotype performed better during the first period and worse during the second period compared to standard breast-yield chickens (Petracci *et al.*, 2013a; Trocino *et al.*, 2015). Indeed, in the second contribution of the present thesis, differences in growth rate between genotypes corresponded to differences in the severity of myopathies with Cobb chickens showing a lower ratet of severely white striped breasts.

Effect of myopathies on meat quality

Results from the different contributions of the present thesis confirmed previous studies that showed that WS and WB significantly increased meat ultimate pH (Petracci *et al.*, 2013b; Bowker and Zhuang, 2016), especially when both meat abnormalities were simultaneously present (Tasoniero *et al.*, 2016; Zambonelli *et*

al., 2016; Kuttappan *et al.*, 2017). Some authors did not report any difference in lightness index as a consequence of myopathies (Petracci *et al.*, 2013b; Tasoniero *et al.*, 2016; First and Fourth contribution). Differently, other authors found higher lightness in WB than in WS and WS-WB breasts (Mudalal *et al.*, 2015), and in severe-degree WS breasts compared to moderate-degree WS (Sánchez-Brambila *et al.*, 2016). Literature also reports different results for redness and yellowness indexes. According to some authors, WS (Sánchez-Brambila *et al.*, 2016; Tasoniero *et al.*, 2016) or WB (Zambonelli *et al.*, 2016) did not affect their values. Others observed lower (fourth contribution; Petracci *et al.*, 2013b) redness index in normal breasts than in defective ones. Mudalal *et al.* (2015) reported higher yellowness index in WB than in normal meat, with its value increasing with the severity of myopathies and with the simultaneous presence of WS and WB (Kuttappan *et al.*, 2017).

Consistently with the results of the present thesis (all contributions of the present thesis), there is a common consent that myopathies increase meat cooking losses especially in the case of WB and with the simultaneous presence of WS and WB (Mudalal *et al.*, 2015; Soglia *et al.*, 2016b; Tasoniero *et al.*, 2016; Zambonelli *et al.*, 2016). Differently, normal and WS breasts may have similar cooking losses (Tasoniero *et al.*, 2016). In fact, a lower ability of holding and binding water has been measured in WB compared to normal and WS meat (Tijare *et al.*, 2016). Finally, our results and some other studies showed that WB breasts had higher shear force values compared to normal and WS breasts (Chatterjee *et al.*, 2016; Soglia *et al.*, 2017), but others did not find any significant difference according to myopathy presence (present study; Kuttappan *et al.*, 2013; Mudalal *et al.*, 2015).

The degeneration process in defective muscles at a histological level also affect meat chemical composition (Kuttappan *et al.*, 2012a; Bowker and Zhuang, 2016): myodegeneration is associated to lipidosis (especially in the case of WS) and fibrosis (especially in the case of WB) (Petracci *et al.*, 2014; Soglia *et al.*, 2016b; first contribution of the present thesis). In fact, in the fourth contribution, WB exhibited the highest water and the lowest protein content compared to the other breasts as well as higher ether extract content than normal meat. Other authors also found higher moisture, lipid, and collagen content and lower protein and ash content in WB and WS than in normal meat (Soglia *et al.*, 2016b). Some of these changes increased with the WS degree (Petracci *et al.*, 2014) and when WS and WB were simultaneously present (Soglia *et al.*, 2016a). In fact, the protein functionality also decreases in WS meat, which alters protein intrinsic properties and reduces sarcoplasmic protein solubility (Bowker and Zhuang, 2016). The increased fat/protein and collagen/protein ratio of affected meat finally implies a decreased nutritional quality (Petracci *et al.*, 2014).
Indeed, poultry meat has a high nutritional value due to its high protein and low lipid contents as well as its high PUFA rate. As measured in our data, linoleic acid (C18:2 n6) and alfa-linolenic acid (C18:3 n3) contribute to the high UFA rate (Grashorn, 2017). The FA composition of the meat is notoriously influenced by the lipid dietary intake (Liburn, 2017), but few data are available on the effect of myopathies on FA meat composition. Some authors found higher SFA rates in normal breasts than in WS and WB ones (Kuttappan *et al.*, 2012a; Soglia *et al.*, 2016a), consistently with the results of the fourth contributio, whereas others

(Traffano-Schiffo *et al.*, 2017) observed an opposite trend. Soglia *et al.* (2016a) did not find any significant difference in total MUFA or PUFA. Nevertheless, they found that linoleic acid (C18:2 n6) rate was lower in normal compared to WB and WS/WB breasts, and alfa-linolenic acid (C18:3 n3) rate was lower in normal breasts compared to WS/WB breasts. These last findings are consistent with the results of Kuttappan *et al.* (2012a) and those of the fourth contribution.

The increased PUFA content in WS and WB meat reported by several authors on the whole muscle could be associated to sarcolemma damages, inflammatory processes, and consequent myopathy development (Kuttappan *et al.*, 2012a; Soglia *et al.*, 2016a). On the other hand, the increased SFA content in the superficial layer of breasts affected by WS (Traffano-Schiffo *et al.*, 2017) could be explained by the lipidosis that can be observed at this specific level. Further research is necessary to clarify the mechanism and the causes of changes in FA profile of poultry meat affected by myodegeneration.

Shelf-life of abnormal meat

Few data are available on the shelf life of abnormal meat. Thus, the fourth contribution specifically add original information about this topic. Indeed, a first significant interaction was found between meat quality and storage time: water content was higher in wooden breasts than in normal and white striped meat only 24 h after slaughtering, whereas no differences among groups were found at 168 h or 264 h post mortem. Likely, protein denaturation during storage altered the water holding capacity of meat (Hughes *et al.*, 2014; Warner, 2017), which in turn increased water losses and reduced water content of all samples, as described during chilling and frozen storage in lamb meat and pork (Coombs *et al.*, 2017; Medić *et al.*, 2018).

During storage, changes in lightness, cooking losses, and shear force during storage were consistent with changes in water retention capability and myofibrils structure of the meat. The high initial meat lightness and cooking losses likely depend on the high rate of free water in meat; later during storage, meat lost free water by dripping; finally, lightness and cooking losses increased again likely because the water previously linked to myofibrils was released with protein denaturation and degeneration. Myofibrils changes also explains the reduction of meat shear force during storage as observed by previous studies (Soglia *et al.*, 2017; Sun et al, 2018) both in normal and WB meat. In line with our results, Sun *et al.* (2018) also found a similar reduction of the compression force over a 8-d storage time at 4°C in all breast categories (normal, mild and moderate/severe WB), and higher changes in drip loss in moderate/severe WB than in normal breasts over time. As time passed, chicken breasts also changed their FA profile, whereas the increase of SFA rate with storage time may be due to changes in MUFA and, especially, PUFA contents, which are expected to decrease during storage because of lipid oxidation (Wood, 2017; Medić *et al.*, 2018).

As for shelf life, microbial growth parameters of TVC and Pseudomonas ssp. were different between normal and affected meat during storage. In fact, normal breasts had the highest initial TVC value and the shortest

TVC lag phase in comparison to WS and WB breasts which means that WS and WB samples undergone a later microbial spoilage. Therefore, shelf life threshold (7 log10 CFU TVC/g) was achieved sooner in normal breasts (130 h) than in WS (149 h) and WB (192 h). Moreover, Pseudomonas ssp. count was initially lower in normal and WS meat than in WB meat; during storage, normal breasts showed a shorter lag phase (27.4 h *vs.* 60.9 h and 77.4 h) and a lower Pseudomonas growth rate compared to abnormal ones. Then, normal samples exhibited a higher final Pseudomonas spp. count than WS and WB meat. The spoilage threshold proposed by Raab *et al.* (2008) for Pseudomonas spp. (7.3 log10 CFu/g) was achieved first in normal breasts compared to WS and WB (122 h *vs.* 139 h and 154 h). The estimated shelf life obtained in this work for normal and abnormal breasts is consistent with previous data for poultry meat stored under different conditions (chilling at 4°C, freeze chilling, packaging with or without modified atmosphere) (Raab *et al.*, 2008; Patsias *et al.*, 2008).

The microbiological profile of poultry meat affected by myopathies has been little investigated. The only study available to our knowledge (Dalgaard *et al.*, 2018) used storage conditions different from ours. In fact, these authors evaluated microbiological profile of normal and moderate or severe WB breasts after 6 d and 8 d of storage at 4°C in modified atmosphere packaging (70% nitrogen, 30% CO2). Under these conditions, at the end of the storage, TVC on meat surface did not differ between normal and WB meat. Nevertheless, Enterobacteriaceae spp. counts in severe WB samples significantly increased from 6 d to 8 d of storage. According to the same authors, alterations in muscle tissue, moisture content, mobile water fraction, drip loss, and pH affected the microflora growth and composition in WB samples stored in modified atmosphere packaging, favoring Enterobacteriaceae spp. and microflora diversification. In fact, water holding capacity, protein, and collagen quality of the meat are known to affect microbial proliferation (Maxcy, 1981). The growth of some foodborne bacteria (specifically E. coli O157:H7) is dependent on their ability of adhering to muscle fiber extracellular matrix (endomysium, perimysium, epimysium) (Chagnot *et al.*, 2017). Thus, the alteration in muscle tissues of WS and WB meat may modify muscle environment and, consequently, microflora growth and composition (Dalgaard *et al.*, 2018). In our study, also differences in FA composition between normal and affected meat could have played a role. In fact, antimicrobial activities of long-chain UFA compared to long-chain SFA are documented (Zheng *et al.*, 2005). These FAs have inhibitory effects both on Gram (+) and Gram (−) bacteria (Lee *et al.*, 2002), likely due to their action at bacterial cell membranes, and changes in FA composition are under study as a strategy to slow down or prevent food spoilage (Lee *et al.*, 2002; Zheng *et al.*, 2005; Desbois and Smith, 2010).

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CONCLUSIONS

The main objective of this PhD thesis was to investigate feeding and management strategies to reduce the occurrence and severity of emerging myopathies, which are relevant issues for the modern industry, and to study how these alterations can modify the main meat traits. To achieve these goals, different trials were carried out testing the effect of various feed restriction strategies (First, Second and Third contributions), the effect of gender (First and Second contributions), the effect of genotypes (First, Second and Third contributions), the effect of occurrence and severity on meat quality (Third and Fourth contributions) and microbial shelf-life (Fourth contribution). Moreover, the onset of myopathies and muscle fiber degeneration was evaluated during growth at different ages in different trials.

Based on results of the experimental activities, the following main conclusions may be drawn:

I. feeding strategies can be an efficient method to control and reduce the growth rate of chickens and, thus, to reduce the occurrence of muscle fiber degeneration and myopathies, as long as the animals are kept under restriction, but when this regime is interrupted, and the chicks show compensatory growth, the positive effort is lost. Nevertheless, if restriction is performed until the end of the trial, e.g. by reducing photoperiod, or if performed late in the growth, the reduction in occurrence/gravity of these alterations is at the expenses of productive performance.
II. gender has been confirmed to play a fundamental role in poultry production. Males have higher performance but are more prone to show wooden breasts compared to females, whereas differences in white striping are not relevant.
III. all commercial genotypes tested are subjected to myopathies; nevertheless, the lower the growth rate, the lower the occurrence of myopathies.
IV. abnormal meat have lower rheological, technological, and nutritional properties compared to normal meat. However, microbial shelf life under aerobic conditions is longer in abnormal meat compared to normal meat.