NUEVOS CONOCIMIENTOS SOBRE LAS MIOPATÍAS DE LOS POLLOS DE ENGORDE

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New insights on broiler breast myopathies

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SUMMARY: Nowadays most of the world's chicken meat production is merely based on intensive farming of few fast-growing hybrids reaching the slaughter weight in a very short time and having high meat yields. The shift from the sale in whole carcass to ready-to-eat and ready-to-cook products has increased importance of quality traits of both fresh meat and raw meat material used for the manufacture of products. This evolution has led to an extreme modification of the genetic background of modern hybrids which are currently used worldwide for the production of chicken meat. However, during the last decades, these evolutions have certainly favoured the occurrence of a high number of abnormalities that are increasing the meat downgrading rates for fresh market retailing and sometimes decreasing the nutritional, sensory and technology proprieties of raw meat materials used for further processing. Among these, the greater concern is currently towards occurrence of abnormalities characterized by a myodegeneration affecting breast fillets (white-striping, woody-breast and spaghetti-meat) which seems directly or indirectly induced by high growth rate and hypertrophy characterizing modern fast-growing broilers.

Keywords: Chicken meat, quality, abnormalities, appearance, tenderness.

Introduction

The development in industrialization and specialization of broiler meat production chains that took place starting from the end of World War II, has led to a worldwide remarkable increase in both the efficiency and the chicken meat production. In the recent years, the lifestyle changes have also dramatically modified the way in which the poultry meat is marketed and consumed and therefore food technologies have become part of the poultry industry, and today much of the production is marketed in the form of cut-up and processed products (Table 1).

Table 1 Trend of world chicken meat production, progress of broiler performance and evolution of market segments and forms of chicken meat in the US (adapted from NCC, 2017).

Year	Production	Broiler performance					Market segments		Market forms		
	(.000 tons)	*market age* (d)	*market weight* (kg)	*average daily gain* (g/d)	*feed to meat gain* (g/g)	*mortality* (%)	*retail grocery* (%)	*food-service* (%)	*whole* (%)	*cut-up parts* (%)	*further processed* (%)
1945	-	84	1.37	16.36	4	10	-	-	-	-	-
1955	-	70	1.39	19.89	3	7	-	-	-	-	-
1965	9,365	63	1.58	25.06	2.4	6	-	-	78	19	3
1975	16,326	56	1.71	30.46	2.1	5	75	25	61	32	7
1985	27,293	49	1.90	38.79	2	5	71	29	29	53	18
1995	46,352	47	2.12	45.07	1.95	5	58	42	10	53	36
2005	70,259	48	2.44	50.75	1.95	4	55	45	11	43	46
2015	96,338	48	2.83	58.97	1.89	4.8	55	45	11	40	49

As a result, nowadays, most of the world's production is merely based on intensive farming of few fast-growing hybrids rapidly reaching the slaughter weight and having high meat yields. In addition, because of the consumers' preference for breast meat and as a consequence of the developing market of cut-up and processed products, broilers are slaughtered at increased weights. Within this context, as a result of the shift in market form from whole carcass to ready-to-eat and ready-to-cook products, the importance of quality traits of both fresh meat and meat used as raw materials for processed products manufacture has remarkably increased. This evolution has led to extreme modifications in the modern hybrids which are currently selected and used worldwide to produce chicken meat.

Notwithstanding, the differences existing in meat quality among the most popular hybrids (i.e. Ross, Cobb and Hubbard) are very limited if compared to the ones observed among and within the medium- and slow-growing genotypes. Thus, the changes in meat quality traits existing in different fast-growing hybrids mainly arise by farming factors and, especially in recent years, by the pre-slaughter and slaughtering phases. In this regard, it is also well known that some features observed in fast-growing hybrids (i.e., muscle hypertrophy, accentuation of glycolytic metabolism of the muscles, poor thermoregulatory capacity, skeletal and vascular fragility, insufficient vascularisation), might directly or indirectly be induced by selection predisposing to the occurrence of meat abnormalities with increased incidence within the last 30 years (Petracci et al., 2015; Velleman, 2015; Alnahhas et al., 2016; Kuttappan et al., 2016).

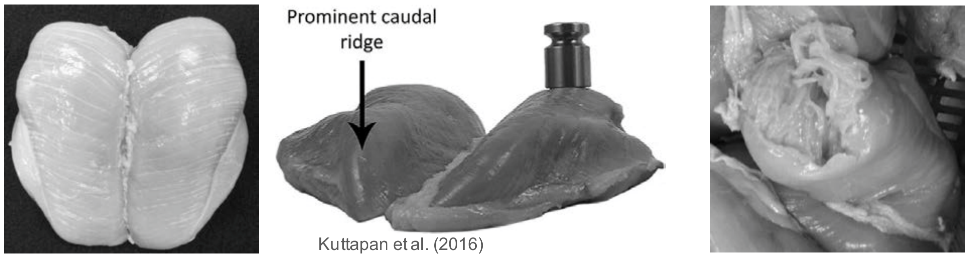
This review is therefore intended to make a summary of the most important qualitative issues affecting the chicken meat of fast growing broilers reared in industrial farming and slaughtering conditions.

Emerging abnormalities as related to myodegeneration

In recent years, a new group of muscle abnormalities characterized by myodegeneration has appeared (Figure 1).

Figure 1. Breast myopathies in broiler chickens.

White striping	Wooden breast	Spaghetti meat



Abnormal white striations parallel to muscle fibres on surface of breast fillets	Macroscopic visible hard, prominent caudal ridge, often associated with paleness and superficial viscous liquid and petachiae	Loose in structure of breast fillets in which it is possible to pull the fibers bundles away with fingers by exerting a moderate pressure

This group includes manifestation of white striations parallel to muscle fibres mainly occurring on the ventral surface of breast fillets (white striping, Kuttappan et al., 2009), myodegeneration of anterior latissimus dorsi (Zimmerman et al., 2012), woody breast condition (often associated with white striping) where muscles are visually hard, out bulging and pale (Sihvo et al., 2014) and poor cohesion of meat or “spaghetti meat” abnormality (tendency toward separation of muscle fiber bundles) (Baldi et al., 2017).

Histological features and possible causative mechanisms

Irrespective of the type of abnormality, the histological features of the skeletal muscles affected by muscular myopathies were found to overlap (Table 2). Indeed, similar histological alterations including profound degenerative myopathic changes (focal and diffuse necrosis) resulting in atrophic fibers which typically loose their cross-striations were found in association with occasional regenerative processes, as proved by the presence of thin fibers exhibiting faint cross-striations and nuclear rowing. In addition, variability in fibers cross-sectional area, proliferation of loose connective tissue and fat deposition (fibrosis and lipidosis) were observed within the endomysial and perimysial spaces in which also interstitial edema and inflammatory cells infiltrates were identified. Similar lesions were also found to affect other muscles composing legs and backs (anterior latissimus dorsi) of the carcass (Zimerman et al., 2012; Kuttappan et al., 2013a).

Table 2 Common and distinctive histological traits of broiler breast myopathies.

Histological traits	Breast myopathy		
	White striping	Wooden breast	Spaghetti meat
Common	Nuclear internalization, loss of cross striations, vacuolar and hyaline degeneration, necrosis and lysis of the fibers, inflammatory cells infiltration, variable cross sectional area (degenerating and regenerating fibers), lipidosis and fibrosis		
Distinctive	Moderate degree of histopathological lesions and deposition of mainly fat (lipidosis)	Severe degree of histopathological lesions and deposition of mainly collagen (fibrosis)	Severe degree of histopathological lesions with progressive rarefaction of the endo- and peri-mysial connective tissue leading to muscle fibers detachment from each other

Recent studies demonstrated that giant, hyaline (hypercontracted) and necrotic fibers might be observed also within the P. major muscles showing no macroscopic lesions ascribable neither to the white-striping nor to the wooden breast defects (Mazzoni et al., 2015; Soglia et al., 2016a; Baldi et al., 2017). Mild-to-severe structural abnormalities were found within the P. major muscles of fast-growing broiler hybrids (Mazzoni et al., 2015). Thus, not only the fillets noticeably affected by muscle abnormalities, but also the macroscopically unaffected cases exhibited structural modifications such as mild myodegeneration in association with the presence of abnormal fibers (Soike and Bergmann, 1998; Mahon, 1999; Mazzoni et al., 2015; Sihvo et al., 2017; Baldi et al., 2017). It is thus clear that a complex aetiology is associated with the occurrence of these recent muscular myopathies and abnormalities affecting the fast-growing genotypes.

Although several histological investigations have been performed to describe the main microscopic processes taking place within the muscles affected by abnormalities, the histological traits are not specific enough to distinguish the different muscle myopathies from each other. In early studies performed by Kuttappan et al. (2013a) and Ferreira et al. (2014), profound degenerative myopathic lesions leading to myofibers degeneration and occasional regeneration, floccular/vacuolar degeneration, mild mineralization and inflammation of the interstitial spaces with edema as well as lymphocites and macrophages infiltrations were associated with the occurrence of white-striping abnormality. In addition, the white-striped muscles simultaneously exhibited polyphasic changes involving muscle fibers fragmentation and phagocytosis, even if proliferation of connective tissue was not evident in broilers slaughtered at earlier ages. The hematologic and serologic profiles assessed on white-striped meat suggested that the occurrence of this muscular abnormality could not be associated neither to infectious or inflammatory mechanisms nor to stress condition. However, the increased levels of creatine kinase in association with serologic enzymes profile observed in white-striped meat are similar to those observed for other degenerative conditions such as stress and muscular dystrophies resulting in muscle damage (Kuttappan et al., 2013b).

Several studies investigated the histological traits of the wooden breast cases (Sihvo et al., 2014; Mazzoni et al., 2015; Soglia et al., 2016a; Sihvo et al., 2017). It could not be possible to identify specific histological patterns for distinguishing between the white-striping and the wooden breast cases. Indeed, polyphasic myodegeneration, rounded fibers and nuclear internalization often associated with lymphocytic infiltrations and occasional regenerative processes were observed within the woody P. major muscles. In addition, proliferation and diffuse thickening of the endomysial and perimysial connective tissue associated with granulation tissue and increased deposition of loose connective tissue (fibrosis) and fat deposition were found to affect the wooden breast fillets (Sihvo et al., 2014; Mazzoni et al., 2015; Soglia et al., 2016a; Radelli et al., 2017). In addition, in agreement with the Kuttappan et al. (2013a), Sihvo et al. (2014) observed vasculitis and irregular perivascular infiltrations of lymphocytes (sometimes disrupting the vascular wall) affecting also the endomysial and perymisial connective tissue of the wooden breast cases (Mazzoni et al., 2015). Since the occurrence of the white-striping and the wooden breast abnormality is frequently combined within the same P. major muscle, Soglia et al. (2016a) investigated whether the simultaneous occurrence of white-striping and wooden breast defects exert an effect on the histological traits of skeletal muscle. A complete reorganization of the skeletal muscle structure involving the replacement of damaged and necrotic muscle fibers (accompanied by interstitial inflammatory infiltrates) with proliferation of connective tissue was found. In addition, as a result of the degenerative and contextual regenerative processes taking place, muscle fibers exhibited rounded profile, variable cross-sectional area and myofibers number (Soglia et al., 2016a). However, both morphological characteristics and histopathological lesions observed within the affected cases were not uniform through the P. major muscle and gradually decrease moving from the external surface towards the inner section (about 1-cm-deep) of the P. major muscles leading to remarkable modification of the muscular architecture (Clark and Velleman, 2016; Soglia et al., 2016a). In a recent study

(Baldi et al., 2017), it was found that spaghetti meat fillets share some microscopic aspects with white striping and wooden breast abnormalities: extensive fiber degeneration and regeneration, hyalinization, poor fiber uniformity, increased fat and connective tissue deposition. A particular characteristic observed in the spaghetti meat histological sections were the progressive rarefaction of the endomysium and perimysium connective tissue. It is likely that the architecture and structural integrity is affected by the immaturity of the newly deposited collagen as previously described by Bilgili (2015).

Within this context, since these muscle myopathies and abnormalities led to similar histological features, a common underlying mechanism responsible for their occurrence might be hypothesised. Thus, different studies have been performed in order to identify the underlying mechanisms at the basis of these alterations. It can be assumed that in case of white-striping abnormality, the increased growth rate (especially of breast muscles) achieved through the selection of fast-growing hybrids might have result in unsustainable pressure on muscle metabolism, overstretching and/or ischemia of the muscular tissue leading to muscle damage and inducing reparative responses. Additionally, the increased free radical production and accumulation of intracellular calcium may alter the integrity of muscle fibre membrane and promote protein degradation through activation of proteases and lipases. This process might lead to degenerative and necrotic processes that overtake the regenerative capacity of muscle thus leading to lipidosis and fibrosis (Kuttappan et al., 2009).

A similar underlying mechanism was hypothesised to be the basis for the wooden breast abnormality. In detail, a higher amount of glycolytic fibers with increased cross-sectional area and reduced capillary-to-fiber ratio was observed within the P. major muscles of fast-growing broilers (Hoving-Bolink et al., 2000). As a result, both muscular oxygenation (hypoxic condition) and metabolic waste products displacement might be impaired leading to an excessive accumulation of reactive oxygen species responsible for oxidative stress and involved in initiating inflammatory processes within the muscle tissue (Mutryn et al., 2015; Zambonelli et al., 2016). Then, complex biological reactions and regenerative processes aimed at contrasting muscle inflammation and limiting necrosis and apoptosis take place. Once more, altered calcium homeostasis and glucose metabolism originate when the degenerative processes resulting from inflammation overtake the regenerative capacity of the muscle tissue. Although to date it is reasonably difficult to define which features of the disease appear primarily and secondarily, a differential expression of several genes is associated with the occurrence of muscular abnormalities. In detail, several genes not only exerting a relevant role in inflammatory processes, extracellular-matrix synthesis (with particular reference to proteoglycans) and muscle development, but also involved in polysaccharides metabolic pathways, glucose metabolism and calcium signalling pathway were up- or down-regulated within the abnormal P. major muscles (Mutryn et al., 2015; Zambonelli et al., 2016). Intriguingly, the increased expression of hypoxia-related genes (Mutryn et al., 2015; Zambonelli et al., 2016) in association with the presence of tubular structures resulting from neovascularization accompanying the myodegenerative processes in affected muscles corroborate the central role exerted by hypoxia in promoting muscle myopathies and abnormalities.

Implications on meat quality features

As expected, the occurrence of muscle myopathies and abnormalities severely affected quality traits and technological properties of meat with the alterations being more pronounced when more than one abnormality coexists within the same muscle.
Within this context, while only a minimal effect is exerted by the PSE-like condition (Qiao et al., 2002), the occurrence of muscle myopathies and abnormalities significantly altered the proximate composition of meat thus affecting its nutritional value. Indeed, if compared o their unaffected counterpart, abnormal muscles

exhibited an overall higher amount of moisture, fat and collagen to the detriment of protein content (Kuttappan et al., 2012; Soglia et al., 2016a; Soglia et al., 2016b; Zambonelli et al., 2016; Baldi et al., 2017). Besides, not only a 3-fold increase in energy deriving from fat but also elevated collagen-to-total protein ratio led to a significantly lowered nutritional value of severe white-striped meat (Petracci et al., 2014; Mudalal et al., 2014). Overall, these differences are likely ascribed to the progressive myodegeneration and regenerative processes, resulting in fibrosis and lipidosis, typically observed within the abnormal muscles. Indeed, while an increased fat (lipidosis) and connective tissue (fibrosis) deposition might respectively account of the higher fat and collagen content, the remarkably elevated moisture level might be attributed to the moderate-to-severe edema resulting from the inflammatory processes (Petracci et al., 2014; Sihvo et al., 2014; Soglia et al., 2016a). Hence, both myodegeneration and the presence of histological lesions may have lead to the extremely reduced protein content observed within the abnormal muscles (Petracci et al., 2014; Soglia et al., 2016a). With regard to minerals content, consistent with the mechanism leading to Duchenne muscular dystrophy in mammal, increased ion levels and alterations in sodium and calcium homeostasis were observed and associated with the development of muscle damage thus promoting the occurrence of muscular abnormalities (Sandercock and Mitchell, 2004; Wallace and McNally, 2009; Soglia et al., 2016a). Wooden breast muscle also exhibited lower content of anserine and carnosine are which are extremely important in homeostasis of contractile muscles as a result of their role as buffering, anti-oxidative, and anti-glycation capacities (Sundekilde et al., 2017). Considering these findings and the relatively low amount of heme pigments observed within the abnormal P. major muscles, the potential pro-oxidant activity of heme-iron released from the globin molecule of a damaged porphirin ring and the contextual exposure of phospholipids resulting from the structural changes associated to the severe myopathic lesions was hypothesised to affect oxidative stability (lipid oxidation and protein carbonylation level) of meat (Soglia et al., 2016a).

Table 3 Meat quality traits of broiler breast myopathies.

Meat quality trait	Type of abnormality			
	Normal	White striping	Wooden breast	Spaghetti meat
Moisture	o	o	+	+
Protein	o	-	--	--
Lipids	o	++	o/+	o/+
Collagen	o	o/+	+	o/+
pH	o	+	++	+
Colour - lightness (L*)	o	o	+	o
Water holding capacity – drip loss	o	o/-	o/-	o/-
Water holding capacity – cook loss	o	-	--	--
Water binding capacity	o	-	--	--
Tenderness	o	o	-	o

o = normal; ++ = much higher; + = higher; - = lower; -- = much lower

With regard to meat quality traits, altered colour and ultimate pH values were observed within the P. major muscles affected by abnormalities. Indeed, as a direct consequence of the strong fibrotic response and the lower amount of heme pigments, increased yellowness and pale colour might be observed in abnormal muscles. If compared to their unaffected counterpart, the affected cases revealed a remarkably higher ultimate pH values which, although associated with a lower glycogen content (Mutryn et al., 2015), were hypothesised to arise from a change in glucose utilization rather that in its availability (Zambonelli et al., 2016; Abasht et al., 2016). Indeed, unexpectedly, although several factors suggest the occurrence of hypoxic conditions, there was not an expected increased conversion of pyruvate into lactate (Zambonelli et al., 2016). Even if high ultimate pH values might significantly increase water holding and processing attitudes of meat, since microbial growth strongly depends on pH, they may compromise the microbiological stability of meat (Barbut et al., 2008). Within this context, it seems reasonable to hypothesise that microbial shelf-life of meat affected by muscle abnormalities could be remarkably reduced as a consequence of their higher ultimate pH values. Moreover, in spite of the higher ultimate pH that should result in improved water holding capacity of meat, the pectoral muscles affected by muscular abnormalities exhibited severely impaired technological properties (marinate uptake, cooking loss and yield), as showed in Table 3. Indeed, reduced water holding and water binding capacities are associated with the occurrence of muscle abnormalities and likely linked to an overall reduction in protein functionality, with more pronounced effect being exerted by the wooden breast rather that the white-striping defect (Mudalal et al., 2014; Mudalal et al., 2015; Bowker and Zhuang, 2016; Tasoniero et al., 2016). This phenomenon might be partly due to protein aggregation and cross-linking following oxidation (Soglia et al., 2016b) and to the overall substantial reduction and altered profile of muscular contractile and sarcoplasmic proteins typically observed within the abnormal muscle tissues (Mudalal et al., 2014; Soglia et al., 2016a; Bowker and Zhuang, 2016).

The occurrence of muscle abnormalities not only alters the visual appearance of meat impairing consumer acceptance (Kuttappan et al., 2012), but also significantly affects its textural properties. Overall, regardless of freshness, cooking and the degree of abnormality, the textural properties of meat, are severely affected by the occurrence of muscle myopathies and abnormalities (Petracci et al., 2013; Mudalal et al., 2015; Soglia et al., 2016a; Chatterjee et al., 2016). However, since extensive poor cohesion (fiber bundles separation) frequently affected the white-striped areas, textural differences were more pronounced with the occurrence of wooden breast rather than white-striping abnormality (Petracci et al., 2013; Mudalal et al., 2015). If compared to their unaffected counterpart, abnormal muscles exhibited elevated compression and Meullenet-Owens razor shear total energy (MORSE) forces as well as increased hardness and chewiness in case of raw and cooked meat, respectively (Petracci et al., 2013; Mudalal et al., 2015; Soglia et al., 2016a; Chatterjee et al., 2016). These changes in textural properties of meat might be explained by the profound alterations affecting the muscle fiber itself as well as the reduced water holding capacity of meat leading to muscle shrinkage and increased packing density of fibers following cooking (Wattanachant et al., 2004; Huff-Lonergan and Lonergan, 2005). On the other hand, the thermally labile cross-links composing the newly deposed connective tissue might contribute to explain the absence of significant differences in the shearing properties of cooked unaffected and affected muscles (Mudalal et al., 2015). Because of undesired appearance and sensory properties, at least breast fillets with severe abnormalities are downgraded by visual evaluation and diverted to manufacture processed products where implications on sensory properties are of less importance. As a consequence, there is interest in developing automated systems to detect abnormal fillets (Yoon et al., 2016).

<u>Conclusions</u>
The genetic selection carried out within the past decades on broiler chickens in order to achieve increased growth rate and breast yield promoted the development of several muscular myopathies and abnormalities. As a consequence, since the affected meat are normally downgraded and devoted to further processing, the occurrence of muscular abnormalities is associated with ever increasing economic losses no longer sustainable by the poultry industry. In addition, not only decreased nutritional properties but also impaired

sensory and technological quality traits have been observed. Within this context, being consumers' more sensitive to animal welfare, the ever-increasing incidence of muscle abnormalities may also negatively consumers' attitude towards poultry meat.

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