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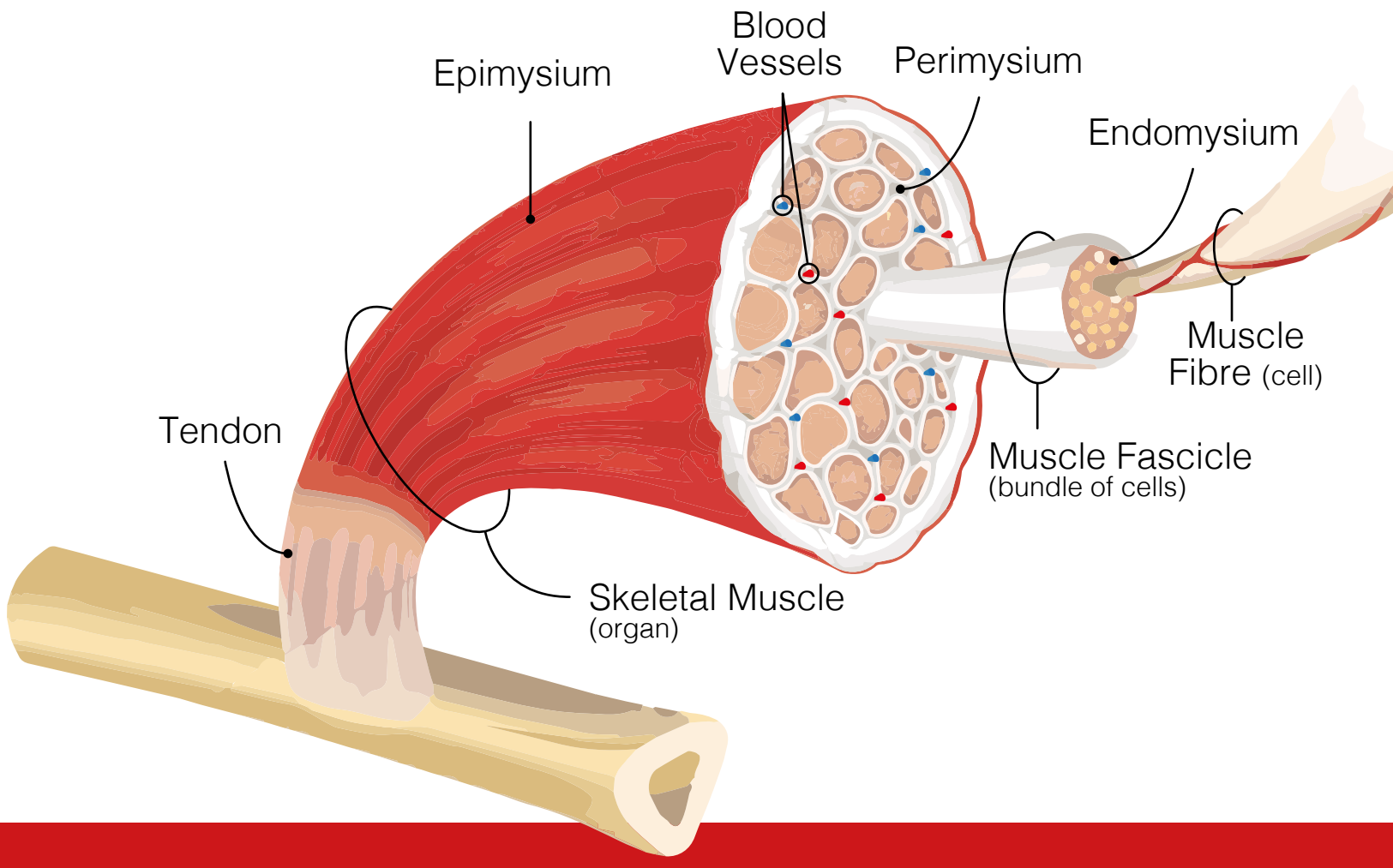

Breast Muscle Myopathies (BMM)

Authors

This document was prepared and reviewed by the Aviagen® Meat Quality Working Group and other independent consultants specializing in this field.

Paul Anton ..... *Sales and Technical Manager for Romania and Republic of Moldavia*
Santiago Avendaño ..... *Director of Global Genetics for Aviagen Group*
Richard Bailey ..... *Poultry Health Scientist*
Sacit Bilgili ..... *Professor Emeritus, Auburn University*
Luis Canela ..... *Area Sales and Technical Manager, Spain*
Alex Corzo ..... *Senior Poultry Nutritionist for North America*
Bryan Fancher ..... *Group Vice President of Global Technical Operations*
Nick French ..... *Global Head of Technical Transfer*
Dinah Nicholson ..... *Global Head, Hatchery Development & Support*
Dan Pearson ..... *Veterinary Health Director*
Lorenzo Rossi ..... *Regional Technical Manger, Western Europe*
Alan Thomson ..... *Managing Director, Aviagen UK Ltd*
Stuart Thomson ..... *Head of Sales & Technical, Aviagen UK Ltd*

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1. Overview

1.1. Actions that can be taken which may reduce incidence and severity of breast muscle myopathies (BMM), in particular wooden breast:

- Focus on good brooding management, good minimum ventilation (do not allow CO_2 levels > 3000ppm), good feed quality and good access to feed.
- Obtain good growth in the first 10 days as this period is important for muscle development: target growth to achieve a body weight of at least 4 times placement weight by 7 days of age.
- Prevent sudden growth acceleration (>120g or 0.26lbs/d), for example after flock thinning.
- Minimize flock disturbances and prevent excessive wing flapping.
- Prevent high body temperatures in the broiler during the mid-and late- growth phase periods. Pay particular attention to temperature at bird level and make sure there is good air movement around the birds. Do not use feed additives that raise body temperature or affect the bird's ability to regulate body temperature.
- Follow Aviagen nutritional recommendations, in particular pay attention to amino acid levels higher than Aviagen advice.
- There is strong evidence that modifying the growth profile can reduce the incidence of BMM although there may be some loss in broiler performance. Modifying the growth curve can be achieved by either:
    - Reducing the quantity of feed provided to the birds to 97% of the feed of ad libitum fed birds and it is recommended that this is done between 15 – 32 days (d).
    - Reducing lysine levels in the diet to 85% of Aviagen advice during the period when birds are growing the fastest (15 – 32d) has been found to significantly reduce BMM with only a small or no effect on live and processing performance.
    - The period of modified growth must take into account the final processing age so that the birds have a sufficient compensatory growth period prior to slaughter. Treatment should not start before 14d and the time applied should equate to the time taken to consume 25% of the expected total feed consumed.
- When feeding all vegetable diets, consider using creatine supplement sources.
- Consider super dosing of phytase by 3 – 6 times the manufacturer's recommendation, the exact increase depending on cost benefit evaluation.
- Use good quality stable fats and oils in diets and use suitable antioxidants in both the ingredients and diets.
- If there is a problem with stringy-spongy muscle then look at scalding temperatures, plucking and carcass chilling practices in the processing plant.

2. Introduction

Over the last ten years there has been an increase in the number of reports of breast muscle myopathies (BMM) observed in the poultry processing plant which, in some cases, can have serious economic consequences for the producer and a negative effect on consumer preference for chicken meat. While the incidence of BMM is erratic and not observed in all regions of the world, it has become an important issue for the industry as a whole. As a consequence Aviagen has invested significant time and resource into researching the subject, as have several universities.

While we still do not fully understand the metabolic causes of BMM, our knowledge has significantly increased over the last five years. The purpose of this document is to summarize what is currently known about breast muscle myopathies, factors that may be involved in causing the myopathy and possible solutions to reduce the incidence and severity. Not all of the myopathies discussed have a major effect on product quality, but are included for completeness.

2.1. History of BMM

The first important breast muscle myopathy to impact the poultry industry was deep pectoral myopathy (DPM), also known as green muscle disease or Oregon disease, which was initially identified in turkeys in the 1960's. DPM manifests as one (or both) of the inner breast fillets (i.e. pectoralis minor) atrophying and turning green. The condition was also seen in broilers in the late 1990s and is still occasionally observed in processing plants.

In the 1990's, two conditions relating to the color of broiler breast meat were described: pale, soft exudative (PSE) and dark, firm, dry (DFD). PSE had initially been observed in pigs and was found to be due to a single gene mutation. However, it was found that PSE and DFD in broilers were not due to a single gene, but primarily due to pre-slaughter stress from the time of catching the birds to go to the processing plant and the actual slaughtering process. Although both PSE and DFD are seen at low levels in most poultry plants and have not resulted in any significant consumer acceptability issues, they do have some minor effects on meat quality.

Since 2010, three BMM have been reported with increasing frequency: white striping (WS), wooden breast (WB) and stringy-spongy (SS), also called spaghetti breast or mushy breast. In most cases, WS has not had a significant effect on consumer acceptance of chicken breast meat, but WB and SS breast, when severe, have resulted in problems within certain products. These myopathies may have been present prior to 2010 but were not recognized in the processing plant.

2.2. Aviagen's response to BMM

While DPM has been part of Aviagen's breeding goal for many years, WS, WB and SS were added to the breeding goal in 2012 with the objective to reduce the genetic propensity to express these conditions in the field.

Aviagen selects against the genetic propensity to breast myopathies within a balanced breeding goal which also includes other traits related to biological efficiency, yield, robustness, welfare and reproductive fitness. Given the low genetic basis of breast myopathies (Bailey et al. 2015) and the time it takes for changes at pedigree level to reach broiler level, it is expected that the genetic

propensity to exhibit these myopathies should have started to reduce in 2018. It should be noted that it is unlikely that the incidence of breast myopathies will reach zero solely due to genetic selection as non-genetic factors also affect the incidence of myopathies **(see Section 7).**

In addition, Aviagen has a multi-disciplinary approach involving nutritionists, veterinarians, geneticists, management specialists and incubation specialists to gather information and coordinate research on the non-genetic factors contributing to the field incidence of all BMM. Aviagen has undertaken a wide range of trials on factors that could affect the incidence of BMM, looking at nutrition, management, incubation practice and genetics. Aviagen has also collaborated with universities and companies undertaking research on BMM. The results from these trials and collaborations will be reported within this document.

3. Description of the BMM issue

3.1. Broiler types affected by BMM

Scientific and field evidence clearly show that BMM can occur in all the modern broiler genotypes in the market place, including slow-growing crosses. The risk of BMM is higher when birds are grown to heavier weights (> 3kg or 6.6lbs) at older ages.

3.2. Distribution of BMM Globally

While these myopathies are being reported in some world regions as a significant issue, the actual occurrence is sporadic and highly variable in incidence when it occurs. The majority of cases have been reported in the EU, USA, Canada, Australia, New Zealand, Japan and Brazil. In several world regions BMM have not been reported as an issue at all. The type of myopathy reported varies among poultry companies – with some reporting WB while others are reporting SS, for example. Currently, the most prevalent myopathy reported is WS.

3.3. Food safety/rejection in the processing plant

The disposition of BMM upon veterinary health inspections during slaughter varies. In general, BMM are considered a quality and not a food safety issue (Bilgili, 2016). To date there has been no evidence of bacterial or viral contamination **(see Section 10).** Most of the product with BMM is sorted and either condemned (DPM) or diverted to alternative uses. However, if the BMM is severe and accompanied by inflammatory signs (i.e., focal pin-point haemorrhages, gelatinous fluid, etc.), then the regulatory authorities may require the condemnation of the entire carcass (Europe) or trimming of the affected areas (Brazil and North America).

3.4. Economic consequences

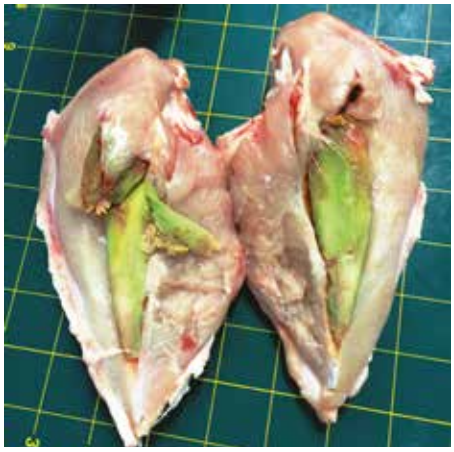
It is possible, but difficult, to estimate the economic consequences of BMM. The amount of muscle trimmed and/or discarded because of BMM can be accounted for if the breast muscles are deboned and an economic analysis can be made on lost product or lost product value. However, there is no practical way to determine the incidence of BMM, and especially of DPM, in whole carcass markets. As for the mild forms of BMM, the sorted product is often diverted to alternative market segments (e.g., further processing) that allows for some value recovery.

4. Description and Histology of BMM

4.1 Deep pectoral myopathy

This condition, first reported in broiler chickens in the 1980's, is more commonly known as Oregon or green muscle disease and is a degenerative muscle process characterized by necrosis and atrophy of the inner breast fillet (i.e. supracoracoideus or minor pectoral muscles). The lesions often impact both inner fillets and vary in color, progressing from a pinkish haemorrhagic appearance to a greenish discoloration **(Figure 1).**

Figure 1. Breast muscle with deep pectoral myopathy

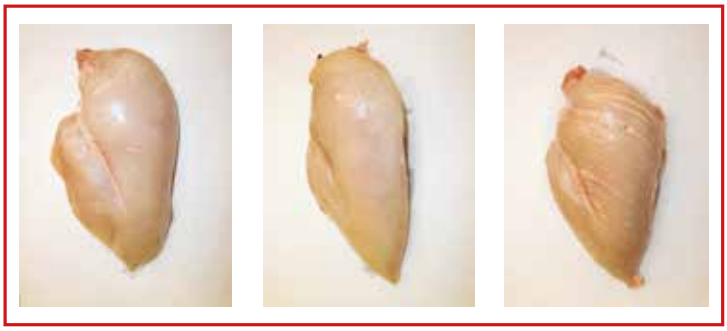


The two pectoral muscles in avian species, the Pectoralis major (outer fillet) and Pectoralis minor (inner fillet or tender), work in synergy to raise and lower the wing. The anatomy of these muscles is, however, intrinsically different in that the inner fillet has a tough outer sheath which is made up of dense fibrous connective tissue and is inelastic. The outer or major muscle is simply surrounded by loose connective tissue that moves easily over the muscle surface as the muscle profile changes. Contraction of the major pectoral muscles and the minor pectoral muscles are responsible for the down- and up-strokes of the wings respectively. During contraction, these muscles expand with increased blood supply (i.e. muscle pumping). The expansion of the minor pectoral muscle, by as much as 25% in volume, is problematic because this muscle is confined in a 'tight compartment', sandwiched between bone (the sternum) and the large breast fillet. The rigid fibrous sheath of minor pectoral muscle restricts increases in muscle volume. Therefore, when intramuscular pressure increases to levels above circulating blood pressure, the blood supply flowing into the muscle stops and, with continued muscle activity, oxygen deficiency rapidly develops, leading to anoxic death (ischaemic necrosis) of the muscle fibres. There is also an additive effect of low muscle pH due to the build-up of lactic acid. In experimental studies, relatively short periods of wing flapping are enough to induce these degenerative changes.

4.2. White striping (WS)

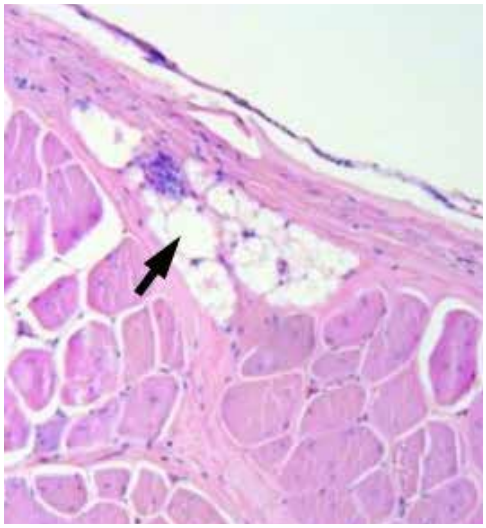
Reports of WS in broiler chickens have increased in recent years. This condition primarily affects the Pectoralis major muscle and is characterized by visible white lines parallel to the direction of the muscle fibres; the quantity and thickness of the white stripes can vary from bird to bird **(Figure 2).**

Figure 2.
Breast fillets with varying degrees of white striping (left to right: mild, moderate and severe).



Histological and chemical analysis of breast muscle displaying WS showed that the white lines are primarily composed of adipose tissue **(Figure 3)**. Research has shown that as the severity of WS increased, the percentage fat as a proportion of dry matter of the muscle increased thus affirming the histological findings of increased adipogenesis (fat deposition) in the tissues.

Figure 3. Histomicrograph of a breast fillet with white striping. The white stripe is composed of adipose (fat) tissue (arrow).

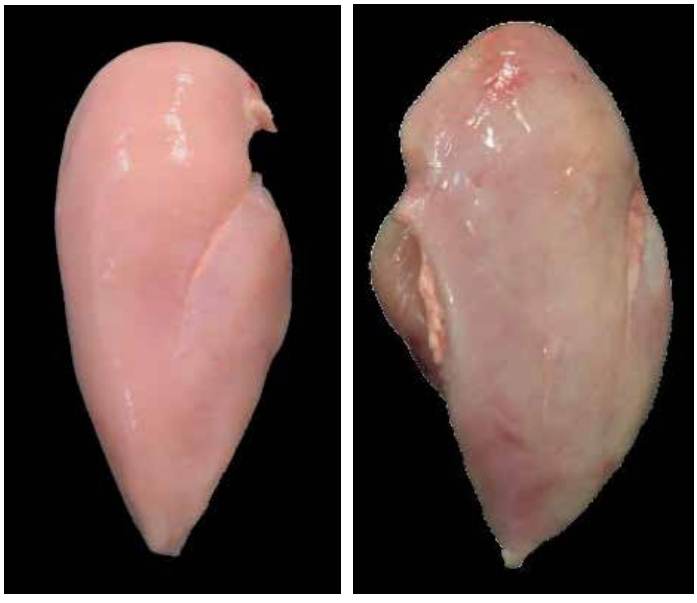


It has been reported that breast tissue severely affected by WS can exhibit an increase in connective tissue with varying degrees of muscle fibre degeneration and regeneration at the microscopic level. All muscle tissue will normally show some level of muscle fibre degeneration and regeneration, but in the case of WS (and also WB) the regeneration process results in fat and connective tissue being laid down rather than a repaired muscle fibre. The exact cause of WS is still not known and understanding this condition is still an active area of research.

4.3. Wooden breast (WB)

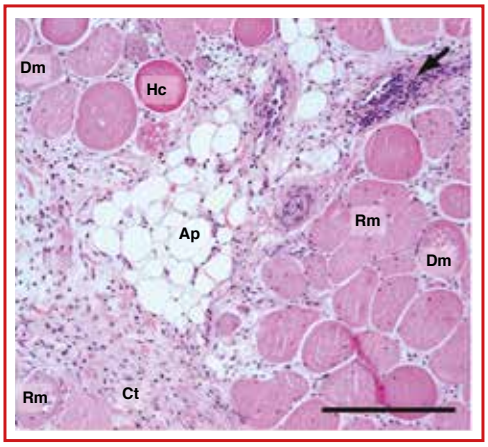
This myopathy also affects the Pectoralis major muscle and is characterized by a hardening of the breast muscle typically in the thicker part of the fillet but the hardening can be found throughout the muscle in more severe cases. Depending on the severity of the condition other visual features of WB include a paler color, surface haemorrhaging and the presence of gelatinous fluid on the muscle surface **(Figure 4).**

Figure 4. Image of a fillet with no wooden breast (left) and a fillet with wooden breast (right).



Histological analysis of the muscle shows active degeneration and regeneration of muscle fibres, hypercontracted fibres, and infiltration of immune cells. Additionally, increased deposition of adipose (fat) and connective tissue **(Figure 5)** can also be seen with the latter thought to contribute to the increased hardness of the muscle. Pathologically WB can be characterized as a myo-degeneration with fibrosis and regeneration.

Figure 5. Histomicrograph of breast muscle with wooden breast. Features of the muscle include degenerating muscle fibres (Dm), regenerating fibres (Rm), adipose tissue (Ap), hypercontracted fibres (Hc), increased connective tissue (Ct) and cellular infiltration (arrow). Black bar shows scale (100µm).



As with WS the exact cause of this condition is not understood however research, discussed later in this document, shows there are many factors which can increase the risk of WB developing.

4.4. Stringy-spongy (SS)

Figure 6. Image of fillet with stringy-spongy.

This condition, also known as Spaghetti or Mushy breast, is characterized by a loss of structural connective tissue, integrity of the breast muscle leading to friability and loosening of the muscle fibres. Broiler chickens are developmentally juveniles and their connective tissues lack the maturity (cross-linking) of adult animals, which is why meat from young animals is tender. Following processing the fibre bundles can separate resulting in the muscle being easily pulled apart by hand **(Figure 6)**. As with the other myopathies, the incidence and severity of SS is variable, ranging from only a small part of the breast being affected to the whole muscle showing the condition.

Histologically the muscle appears disorganized in structure, with a mix of very small and very large muscle fibres **(Figure 7)**. There is evidence of hypercontracted muscle fibres and active degeneration and regeneration of muscle fibres; although this is less marked compared to WB. This condition is not as well understood as WB and Aviagen is now working to better understand SS and how it could possibly be alleviated or minimized.

Figure 7. Histomicrograph of breast muscle affected by stringy-spongy. Features of the muscle include large (Lg) and small (Sm) muscle fibres along with hypercontracted fibres (Hc), (100µm).

There is ongoing research into this condition, but it is thought to be linked to an increase in lactic acid accumulation in the muscle which can cause degradation of the connective tissue holding the muscle fibres and bundles together. Additionally, increased levels of lactic acid can also inhibit protein synthesis which may also impact upon the maturation and thus integrity of the connective tissue in the muscle. Alternatively, low muscle pH resulting from lactic acid accumulation can stimulate proteolytic enzymes to degrade the developmentally labile connective tissue. A third possible cause or contributing factor is inadequate dietary levels of amino acids (AA) critical for proper development of the connective tissue "sheath" (i.e. endomysium) covering the muscle fibres (e.g. proline) – particularly when birds are fed plant-protein based diets. In general, plant-based feed ingredients are much lower in proline than are animal proteins; hence the possible risk factor associated with feeding only plant-based diets.

5. Impact of BMM on eating quality

Table 1 summarizes the effects of different BMM on product quality and usability in the processing plant. As noted earlier, BMM are a food quality issue, and not a public health issue. The authorities in both the USA and UK have clearly stated that this is not a public health issue but BMM may require some degree of rejection if severe. FSAops, the UK's control body for post mortem inspection rejects, has produced a condition card that is a useful reference on how to deal with WB at processing. More details are provided in the rest of this section.

Table 1. The effect of breast myopathies on product quality

Breast Myopathy	Affected Breast	Rest of Carcass	Comments
White Striping (WS)	In most cases used normally Very severe WS may not be able to be sold as breast fillets	Can be used	Increased fat content of the breast may create problem for maximum fat content labelling Consumer preference for no striping
Wooden Breast (WB)	Severe WB cannot be used for breast fillets and must be converted into another product where toughness is not an issue	Can be used	Reduced water holding capacity (WHC) Reduced marinade uptake Higher drip loss Increased cooking loss
Stringy–Spongy (SS)	Cannot be used in products that require normal muscle structural integrity	Can be used	Higher moisture content Difficult to slice
Deep Pectoral Myopathy (DPM)	Condemned breast fillets	In most cases can be used when trimmed away from affected meat	Particularly problematic when selling whole birds as the myopathy can remain undetected until the consumer cuts the whole bird after purchase
Pale, Soft, Exudative (PSE)	Can be used	Can be used	Consumer preference for no PSE, but not strong Low WHC Pale Color
Dark, Firm, Dry (DFD)	Can be used	Can be used	Shorter shelf life High WHC

5.1. White striping (WS)

There is no compelling reason why the consumer cannot eat chicken breast with WS. There is no evidence of infectious agents in the meat (Kuttappan et al., 2013b) and the only significant difference is slightly higher fat and collagen content (Petracci et al., 2014). Although it has been claimed that there is a decline in the nutritional value of breast meat with WS due to fat content increasing by 224% (CIWF, 2016), this claim needs to be put into perspective as chicken breast with WS is still very low in fat and high in protein compared to other meat sources **(Table 2)**.

Table 2. Fat and protein content of raw meats

Meat	% Fat	% Protein	Reference
Chicken breast (w/o skin) *No striping*	0.8 – 1.0	22.8 – 22.9	Petracci et al. (2014), Mudalal et al. (2014)
Chicken breast (w/o skin) *Moderate striping*	1.5	22.2	Petracci et al. (2014), Mudalal et al. (2014)
Chicken breast (w/o skin) *Severe striping*	2.2 – 2.5	18.7 – 20.9	Petracci et al. (2014), Mudalal et al. (2014)
Pork chop	6.9	21.5	USDA Foods database
Lamb chop	4.9	20.4	USDA Foods database
Beef sirloin	6.4	21.8	USDA Foods database
Salmon	10.4	19.9	USDA Foods database

Studies investigating the eating qualities of breast meat with WS have not shown consistent differences. Kuttappan et al. (2013a) found no differences in eating quality, while other studies found an increase in cooking loss and marinade uptake (Petracci et al., 2013; Mudalal et al., 2014, 2015). Some studies have shown a lower shear force (indicating more tender meat) in breasts with severe WS (Petracci et al., 2013) whereas a taste panel study found no difference in juiciness but breasts with WS to be slightly harder and chewier (Brambila et al., 2016). A study by Kuttappan et al. (2012a) has shown that consumers prefer breast fillets without WS and acceptance decreased as the severity of WS increased. However, in no instance were breasts with WS found to be unacceptable.

5.2. Wooden breast (WB)

Severe WB (greater than score 1) has a marked increase on the texture and chewiness of the meat when measured either by texture analyser (Mudalal et al., 2014; Chatterjee et al., 2016) or by a taste panel (Tasoniero et al., 2016). WB samples also had a higher ultimate pH, lower water holding capacity, reduced marinade uptake, increased drip loss and increased cooking loss (Mudalal et al., 2014; Dalle Zotte et al., 2014; Soglia et al., 2015).

Chicken producers with a high incidence of WB do see an increase in customer complaints when these breasts are used in certain products. In most cases, WB meat is used for comminuted products where toughness is not a concern.

5.3. Stringy-spongy (SS) or spaghetti breast

Only one study has investigated the effect of SS breast on meat quality (Baldi et al., 2017). These authors showed that the SS meat had higher moisture content and lower protein content than normal meat. In processing plants the poor structure of the SS breast means it can only be used for limited products and has a lower market value.

5.4. Pale, soft and exudative (PSE)

Droval et al. (2012) showed that consumers preferred normal breasts to PSE breasts, but only identified flavor as significantly different. In a study by Desai et al. (2016), PSE in breast meat was found to be less tender and juicy than normal breast meat when evaluated by a taste panel. While 81% of the taste panel like the taste of normal breast meat only 62% of the panel liked the taste of PSE meat.

While there are measureable differences in eating quality between normal and PSE breasts, it is not usually seen as a reason for excluding PSE meat from certain product categories.

5.5. Dark, firm and dry (DFD)

Breast fillets with DFD have been shown to have a shorter shelf life (Allen et al., 1997). There is no research on consumer preference or eating quality of DFD breast meat.

5.6. Deep pectoral muscle myopathy (DPM)

The muscle fillet affected by DPM is rejected for human consumption and the remainder of the carcass may be trimmed to recover the unaffected meat. The largest issue is for the whole bird market where the problem is found by the consumer.

6. Basic muscle biology

6.1. Structure and function

Muscle tissue is comprised of 75% water, 20% protein, and the remaining 5% of fats, carbohydrates and minerals. Skeletal muscles attach through bundles of collagen (tendons) to the bones of the skeleton to facilitate body structure, posture and movement, and thermoregulation. The skeletal muscle is composed of bundles (fascicles) of muscle fibres covered by layers of connective tissue sheet (endomysium, perimysium and epimysium) which is composed of collagen and other glyco-proteins **(Figure 8)**. The connective tissue is an important component of muscle as it contains the blood and nerve supply, and also provides structural integrity to the muscle.

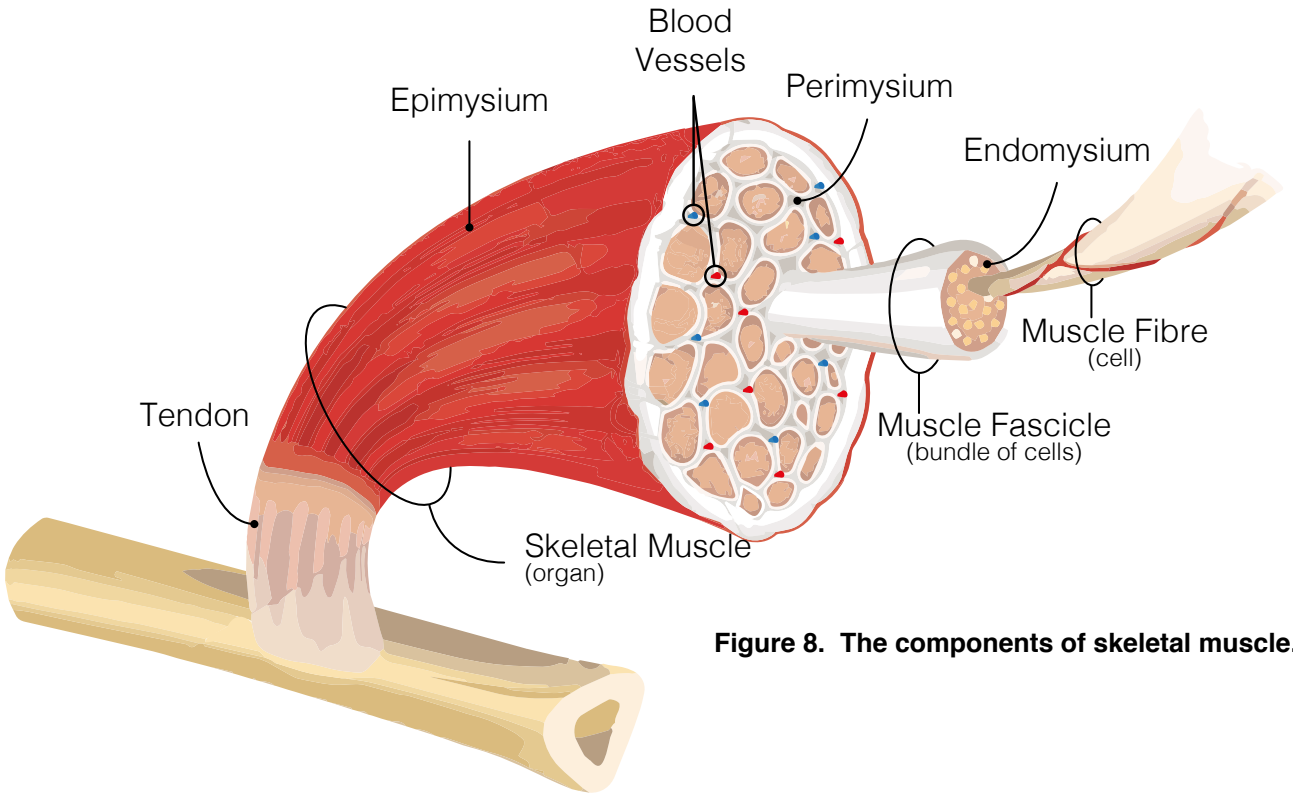


Figure 8. The components of skeletal muscle.

Muscle fibres are in turn composed of contractile thick (myosin) and thin (e.g. actin, troponin, tropomyocin) protein filaments of myofibrils which slide over each other during muscle contraction. Contraction of the muscle is triggered by a nervous signal (action potential) that ultimately leads to an increase of calcium levels in the muscle cells, which in turn initiates the movement of the myofibrils (contraction). The extent of contraction (speed, force and duration of tension) is regulated by separate and voluntary nervous control of multiple bundles of muscle fibres within the muscle. Muscles are usually arranged in opposition so that when a group of muscles contract, the opposing muscles relax or lengthen.

Muscle contraction requires large amounts of energy however muscles store only enough energy to initiate muscle contraction and, once used, energy for subsequent contractions must be generated by the muscle. Muscles can produce energy through aerobic (oxygen dependant) metabolism of

fatty acids or by anaerobic (oxygen independent) metabolism of glucose. Nutrients for the production of energy by muscle cells can either come from the blood stream or from the energy (glycogen) stored in the muscle. At periods of peak muscular activity, oxygen cannot diffuse into the muscle fibre fast enough for aerobic metabolism to continue and anaerobic metabolism dominates. The final product of anaerobic metabolism is lactic acid, which can accumulate in the muscle lowering pH and inhibiting the mechanisms for contraction (muscle fatigue). During muscle contraction, energy reserves are consumed resulting in excess heat and lactic acid production. Upon rest, the conditions within the muscle return to normal with the removal of lactic acid, replacement of energy reserves and dissipation of heat.

Skeletal muscles can differ in their metabolism and maybe further categorised broadly as “Red” or aerobic (dense with capillaries and rich in mitochondria and myoglobin) and “White” or anaerobic (few capillaries, little or no mitochondria and myoglobin). Most muscles contain a mixture of fibre types. The pectoral muscles (major and minor) of chickens are composed of white muscle fibres, which contract fast, rely primarily on stored glycogen for energy and fatigue easily with the build-up of lactic acid. The leg muscles are made up of mostly red muscle fibres that contract slowly, but can utilize fatty acids in addition to glycogen as energy source, and therefore sustain prolonged aerobic activity.

6.2. Muscle growth and development

Muscle cells are formed in the embryo through the process of myogenesis; muscle precursor cells fuse together to form long muscle cells which become the muscle fibres. Embryonic muscle increases in size through proliferation of newly formed muscle cells which increases the number of muscle fibres (hyperplasia). After hatch, the muscle only increases in size though the enlargement of the muscle cells (hypertrophy). Muscle fibre growth is dependent upon recruitment of specialized precursor cells called satellite cells which sit on the surface of muscle cells; these cells proliferate and fuse into the muscle cell and provide additional DNA for muscle growth. Inadequate early chick nutrition can be detrimental to early proliferation of satellite cells resulting in a decreased capacity of the muscle to grow later in life (Velleman et al., 2010). The growth of muscle fibres requires considerable accretion of structural and contractile tissue proteins; however, the contractile muscle proteins have limited life and must be broken down and resynthesized (protein turnover). In young animals, 20-25% of the proteins are degraded and replaced daily. The synthesis and breakdown of proteins are controlled by complex cellular mechanisms and influenced by many factors such as age, disease, over- and under-nutrition, exercise, inactivity, endogenous and exogenous agents, and genetics.

6.3. Muscle repair

Muscle cells undergo continuous maintenance as part of normal cellular function. Impairment of normal muscle structure and function can occur from both physical (strain, micro-tears, and trauma) and chemical (altered cellular pH or oxidative damage) insults. Muscle repair is a natural physiological process requiring the activation, proliferation and recruitment of satellite cells, and involves a cascading sequence of cell-signalling molecules, hormones and growth factors. The satellite cells proliferate and the new cells fuse together to repair the damage and form new myofibres. The ability of a satellite cell to divide and proliferate is finite which means their activity does decrease with age. For this reason, failure to establish an adequate number of these cells in the young chick may impact the muscle’s capacity for repair later in life. It is important to note that higher numbers of satellite cells are found in red muscle fibres compared to white muscle fibres, as red muscle undergoes more maintenance and repair from daily activities.

6.4. Muscle changes post-slaughter

Upon death there is a change in intramuscular metabolism as blood supply to the muscle tissue ceases; this interrupts the flow of oxygen and energy to the tissue and removal of waste metabolites. Uncontrolled nerve impulses to the muscle tissue cause a large release of calcium into the muscle cells which activates various proteolytic enzymes. As the level of oxygen in the muscle falls there is a switch to anaerobic metabolism to generate energy from stored muscle glycogen. This consequence, combined with the interruption to blood flow, results in accumulation of the lactic acid in the muscle, lowering tissue pH. The post mortem degradation of muscle structure is primarily due to the activation of proteolytic enzymes (calpains, calpastatin and cathepsins). The degradation of muscle ultrastructure is an important factor in tenderness of meat upon aging, once the animal is slaughtered. The amount of lactic acid produced post mortem depends upon the level of glycogen in the muscle (influenced by pre-slaughter activity) and the rate of cooling (lactic acid production and activity of proteolytic enzymes occurs only when muscle cells are warm).

6.5. Physiological changes that may result in BMM

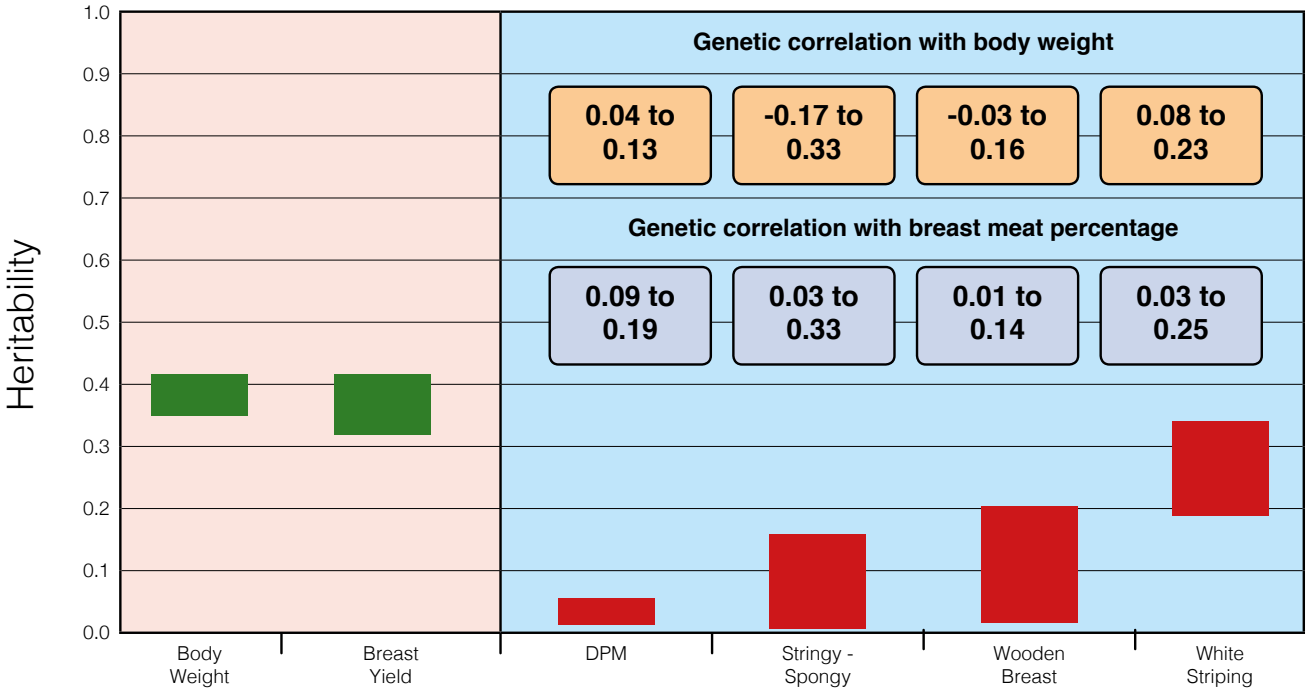
The exact cause(s) of BMM are yet to be identified. Breast muscles of broiler chickens have unique structural and developmental features that increase their susceptibility to cellular damage by ischaemia. Ischaemia begins when the local blood flow cannot satisfy the metabolic demands of the muscle tissue (the rate of oxygen supply and removal of metabolic waste-products like lactic acid). This may be especially critical during periods of high metabolic activity (e.g. exertion or over-stretching of breast muscle). The build-up of lactic acid and lack of sufficient oxygen can result in loss of muscle cell membrane integrity, depletion of energy and increase in intracellular calcium leading to skeletal muscle cell hyper-contractility and, ultimately, cell death. When there is damage to a muscle fibre the resultant breakdown products trigger a natural local inflammatory response for the purpose of clean up and repair. The muscle repair process includes the deposition of large amounts of connective tissue. Collagen, the primary protein in connective tissue, is initially deposited as a pro-collagen which is of a less stable nature than mature collagen with cross-links. The freshly deposited pro-collagen is more susceptible to pH and temperature effects and is likely at higher risk for myopathies like SS.

Heat load (exogenous or endogenous heat) and oxidative damage may play a role in the development of BMM. These factors can result in prolonged contraction, muscle rigidity and ischemia. As a result of this process, reactive oxygen species (ROS; superoxide anions) are increased. ROS production causes oxidative damage to proteins and DNA within the muscle. ROS also decrease the mitochondrial calcium release channel and the ability of mitochondria to produce energy. The resulting calcium overload leads to increased glycolysis and lactate production. Several pharmacological and phytogenic substances (e.g. some antibiotics; thymol) stimulate heat stress and increase ROS levels. Also, recent research indicates that some satellite cell populations in the breast muscle can differentiate into adipocytes at high temperatures (Clark et al., 2017), which may be important in the development of WS.

7. Genetics of BMM

Current published research indicates that myopathies can be observed in a number of commercial broiler strains differing in breast yield and growth rate (Kuttappan et al., 2012b, c, d; Petracci et al., 2013; Shivo et al., 2013; Ferreira et al., 2014; Mudalal et al., 2015). As part of the Aviagen breeding program, the incidence and severity of myopathies have been recorded on individual selection candidates in multiple pedigree lines for several years. Using these records, the genetic basis of the myopathies can be characterized by estimating heritabilities for myopathies and their genetic relationships with growth rate and breast muscle yield (Bailey et al., 2015). The range of heritabilities of breast muscle myopathies are displayed, along with those for breast yield and body weight for comparison, in **Figure 9.**

Figure 9. Heritabilities of BMM, body weight and breast yield (Bailey et al., 2015).

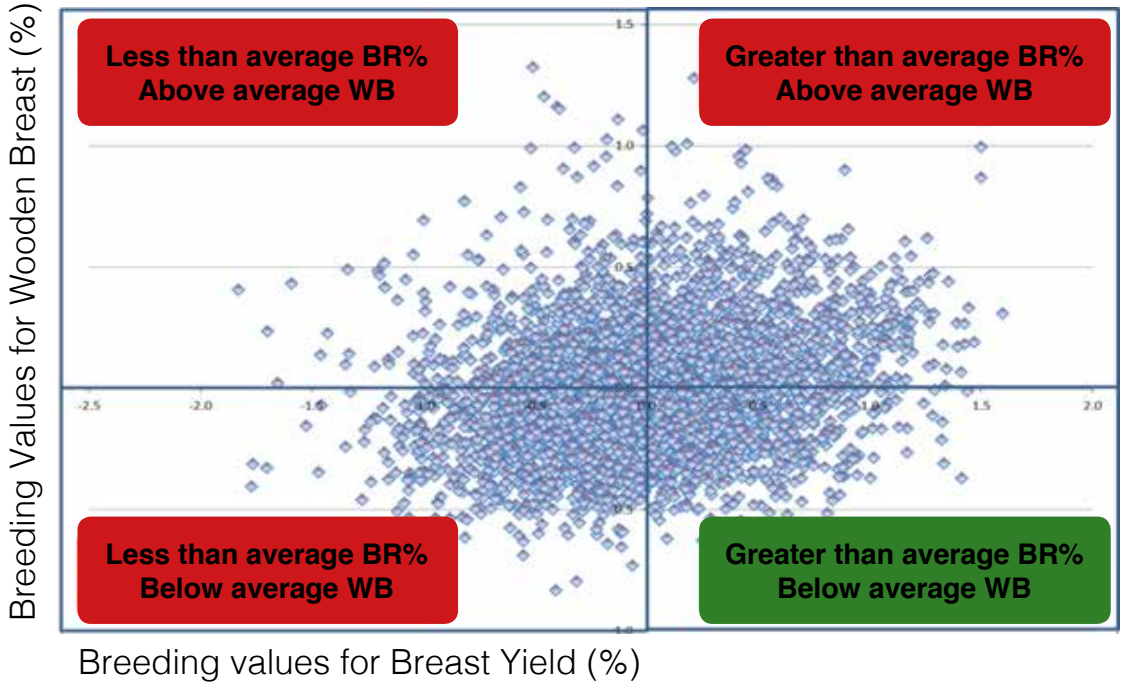


Every trait in the chicken is under the control of genetic factors (passed down from the parents) and environmental factors (management, nutrition, gut and immune challenge, house environmental conditions, climate, etc.) and the sum of these effects results in the observed field performance. Heritability is the proportion of the observed variation in the trait that is explained by genetic effects.

Figure 9 compares the range of heritability estimates of body weight and breast yield (green bars) with those of BMM (red bars). For example body weight and breast yield have heritabilities ranging between 0.32 and 0.42 which are higher than those for the BMM, indicating the influence of non-genetic factors is much greater for BMM. The heritabilities for deep pectoral myopathy, stringy-spongy and wooden breast are very low ranging between 0.02-0.20, this indicates that the non-genetic factors explain at least 80% of the variation in these traits. The heritability for WS ranges from 0.19-0.34. This shows that there is a larger genetic component to the expression of WS compared to other BMM; however, there is still a predominant influence of non-genetic factors explaining 66%-81% of the variation in WS.

Genetic correlation is a measure of the shared genetic basis for two traits; i.e. does genetic selection for one trait result in a correlated genetic response in another trait. The chart in **Figure 9** shows the genetic correlations between body weight and breast yield with BMM. Body weight was estimated to have a genetic correlation between -0.170-0.228 with BMM, and breast yield a genetic correlation of between 0.092-0.330 with the BMM. The low genetic correlations described indicate there is a low level link between increased breast meat yield and body weight, and expression of BMM. While it has been suggested that the incidence of breast muscle myopathies is linked to genetic selection of birds for increased breast yield and increased growth rates, our estimates of genetic correlations indicate that selection for these traits does not necessarily mean increased risk of BMM. The scatter plot below **(Figure 10)** gives the breeding values for WB and % breast yield. It shows that WB can occur in birds with both high and low % breast yield. It can be seen that there are a proportion of birds with a high genetic potential for % breast yield and below average WB in the bottom right quadrant of the plot. This proportion of birds represents those which can be selected for both traits in the desired direction as part of a balanced breeding strategy. Aviagen continues to address BMM as part of a balanced breeding program aiming to reduce the genetic propensity to express BMM in the field. Given the relatively low genetic basis of BMM, genetic selection will yield small to moderate improvements in the expression of BMM. It is clear that the key to reducing the expression of BMM will be a better understanding of the effects of environmental factors, such as management or nutrition, on their expression. More research is required on the non-genetic factors and their impact on BMM.

Figure 10. Scatter plot showing breeding values for wooden breast (WB) and breast meat yield (BR%).



7.1. Gene expression studies

One method for understanding the cellular mechanisms involved in the development of conditions such as WB involves studying gene expression. In birds exhibiting WB this method measures whether genes are up-or-down regulated in affected compared to unaffected birds (i.e. are certain biological pathways more or less active). There has been a number of gene expression studies showing changes in gene expression in birds with WB compared to those birds without the condition (Mutryn et al., 2015; Zambonelli et al., 2016).

Results from these studies show that muscles with myopathies have an increased expression of a range of genes associated with metabolic (hypoxia, oxidative stress, calcium metabolism, fat

metabolism, inflammation), anatomical, and structural biological processes. These cellular processes confirm what is seen in histopathological examination of muscles with the myopathies - namely, fibrosis, immune cell infiltration, hypoxia, fat deposition and muscle fibre degeneration and repair. The work by our collaborator Dr. Sandra Velleman (Ohio State University) indicates that breast muscle with WB has increased expression of genes linked to satellite cell proliferation and differentiation which are indicative of active muscle growth and repair (Velleman, personal communication). Furthermore, this research has shown increased expression of genes involved in collagen alignment and cross-linking which results in a more rigid muscle structure.

Through the study of gene expression we are able to better understand the pathophysiology of myopathies; however, it must be noted that they do not necessarily offer an explanation of cause and effect (i.e. are these results demonstrating cause and effects or simply consequences associated with presence of the myopathy). While molecular approaches to understand the pathophysiology have revealed differences in many metabolic pathways, it is not feasible to incorporate all these interactions into a practical breeding program due to the complex nature of these pathways. However, the effects of all the underlying genes identified in gene expression studies can be captured by identifying birds with BMM. The information on the genetic basis and genetic correlations of the BMM with other traits can then be included as part of a balanced breeding goal as described above.

7.2 Metabolomic and proteomic analysis

Further investigations to understand the underlying mechanisms of BMM include the use of metabolomics and proteomics to characterize the cellular and physiological differences between breast fillets with and without myopathies. Kuttappan et al. (2017) demonstrated, through the use of proteomics, that breast fillets with WB have significant differences in proteins related to cellular movement, carbohydrate metabolism, protein synthesis and protein maturation compared to breast muscles without WB. Boerboom et al. (2018) analysed breast fillets with and without WS using metabolomics to identify biological pathways which may explain why WS occurs. Significant differences were found in carbohydrate metabolism and fatty acid composition of the WS fillets compared to fillets without WS, and there was also evidence of hypoxia and oxidative stress within the affected fillets. The results from both the metabolomic and proteomic analyses are in agreement with the findings in the previously mentioned gene expression studies. These studies show differences in the composition and physiology of fillets with myopathy which is expected as the histological analysis shows evidence of structural and biochemical changes in breast muscle with myopathy. While these studies show what is occurring within the muscle tissue at the time of sampling and allow for the speculation of potential causes for the cause of the myopathies, it is still unclear as to what is causing the initial disruption within the tissues and more research is needed.

8. Growth curve and BMM

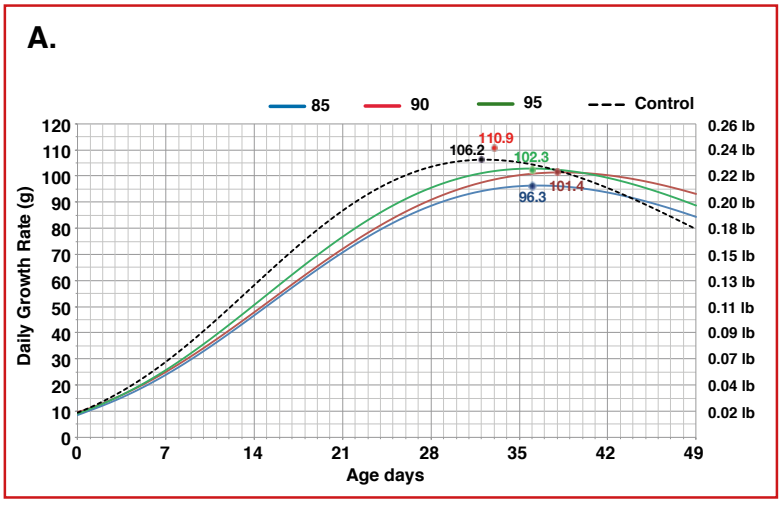
In 2014, field observations from Western Europe suggested that sudden growth accelerations such as those occurring post-thinning (which results in abrupt increases in available feeder, drinker and floor space for the remaining birds) increased the incidence and severity of WB. Around the same time, a number of customers, academics and media representatives anecdotally attributed BMM to the fast growth rate of modern broilers.

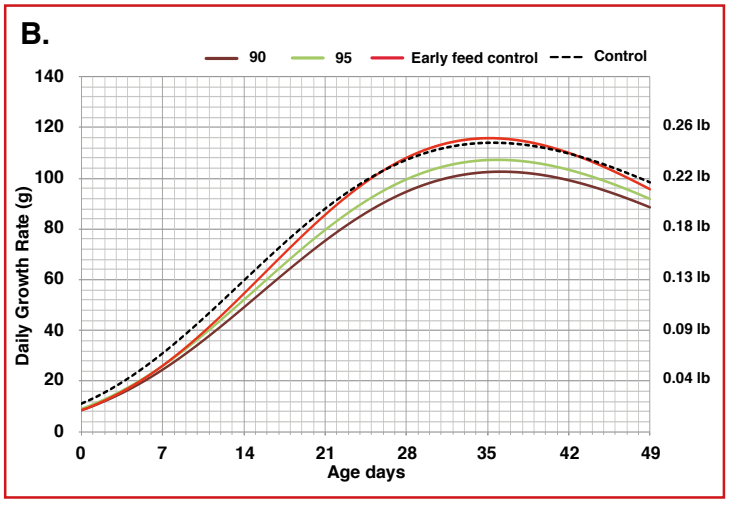
With little scientific evidence being available to support or refute this claim, Aviagen and Auburn University began a collaborative PhD research project to gain better understanding of the impact of the broiler growth curve per se, and modifications to the growth curve, on the incidence and severity of BMM.

8.1 Modifying growth using quantitative feed control

The effect of controlling feed intake and thereby modifying the growth curve on the incidence on BMM in high yielding (Ross® 708) male broiler chicks was investigated in two studies (Meloche et al., 2018a; internal Aviagen trial). These studies fed the chicks either ad libitum or between 80 – 95% of the control ad libitum treatment throughout the growing period. In both trials the birds on the controlled feeding treatment had lower body weights at the final processing age and a reduction in the incidence of both WB and WS **(Table 3, Figure 11).** Meloche et al. (2108a) concluded that controlling feed by 95% of ad libitum throughout the life significantly reduced BMM with no further statistically significant improvement beyond that level of quantitative feed control, and these results were supported by results from the internal Aviagen trial. In all cases the final processing body weight was reduced and in the internal Aviagen trial FCR was also increased when feed intake was controlled **(Table 3).**

Figure 11. The effect of quantitative feed control on daily growth rate in (a) Meloche et al. (2018a) and (b) internal Aviagen trial.





Meloche et al. (2018a) also recorded levels of creatine kinase (CK) and lactate dehydrogenase (LDH); these two enzymes are general indicators of muscle and cell damage. Compared to the ad libitum fed birds plasma levels of both CK and LHD were reduced in birds given 95, 90 or 85% of ad libitum feed intake. Although multiple isoforms of CK are expressed in various tissues, the major isoform present in chicken plasma originates from skeletal muscle. Plasma CK has been previously utilized as an indicator of compromised membrane integrity in the presence of myopathies. If blood CK levels are elevated, it is indicative of muscle damage. LDH is another intracellular enzyme which catalyzes conversion of pyruvate to lactate – a key pathway in glycolytic muscle tissue as is the case with chicken breast muscle. LDH is released into the bloodstream when cells are damaged or destroyed. Thus, both of these enzymes can be used as general indicators of tissue and cellular damage.

Studies have also investigated controlled feeding for part of the growth curve. Trocino et al. (2015) found an increase in WS and no change in WB when broilers were control fed 80% of ad libitum between 13 – 21 days of age and processed at 46 days of age. Similarly, feeding 95% of ad libitum from 0 – 11 days of age had no effect on either final live weight or WB and WS (**Table 3**, internal Aviagen trial). These studies suggest that controlling feed intake for only part of the growing period (0 - 11d) is not as effective in controlling BMM as reducing intake for the whole growing period.

These studies have shown that it is possible to reduce the incidence of WS and WB by modifying the growth curve per se. Where farms are equipped with equipment that can measure and control feed intake, it can be a method of reducing the incidence of BMM, although this will have to be balanced against a loss in broiler performance if the controlled feeding is applied to the whole growth curve. As noted in **Section 8.3**, it may be possible to control feed for a specific period to reduce BMM without negatively impacting broiler performance. Some producers have successfully reduced the incidence of WB by reducing feed intake to 97% of ad libitum using this method. However, most farms do not have the equipment to control feed the broilers and alternative methods of controlling the growth curve are required.

Table 3. Summary of controlled feeding trials[1].

Study	Processing Age (d)	Treatment	WB score[2]	WS score[2]	BW kg (lbs)	FCR	FCR Adj to 3.5kg/7.7lbs[3,4]
Meloche et al. (2018a)	33	Ad libitum Control 95% of ad libitum 90% of ad libitum 80% of ad libitum Linear[5] Quadratic	0.643 0.107 0.143 0.107 p<0.001 p=0.01	0.786 0.500 0.429 0.250 p<0.01 ns			
	43	Ad libitum Control 95% of ad libitum 90% of ad libitum 80% of ad libitum Linear Quadratic	1.593 0.536 0.107 0.143 p<0.001 p<0.001	1.851 1.321 1.000 0.964 p<0.001 p=0.03			
	50	Ad libitum Control 95% of ad libitum 90% of ad libitum 80% of ad libitum Linear Quadratic	0.702 0.393 0.143 0.143 p<0.001 ns	1.593 0.857 1.071 0.821 p<0.001 p=0.01	3.748 (8.26) 3.579 (7.89) 3.516 (7.75) 3.347 (7.38) p<0.001 ns	1.823 1.785 1.725 1.714 p<0.001 ns	1.740 1.741 1.712 1.740
	Processing Age (d)	**Treatment**	**WB score**	**WS score**	**Days to achieve 3.35kg/7.39lbs**	**FCR**	**FCR Adj to 3.35kg/7.39lbs[3]**
Internal Aviagen Trial	32	Ad libitum Control 95% of ad libitum 90% of ad libitum 95% of ad libitum until day 11 only	0.05 0.00 0.00 0.00 ns	0.00 0.14 0.08 0.20 ns			
	49	Ad libitum Control 95% of ad libitum 90% of ad libitum 95% of ad libitum until day 11 only	0.23 0.13 0.10 0.27 p<0.05	0.74 0.50 0.44 0.61 p<0.001	43.6 46.4 48.2 44.4		1.548 1.658 1.675 1.516

1. Body weight and FCR only reported at final processing age. Breast meat yield not reported. 2. Breast fillets were scored for the presence of myopathy as either 0 (none), 1 (mild) or 2 (severe). Myopathy score is the average score for all the fillets evaluated. 3. FCR Adj: FCR Adjusted to stated weight. 4. Adjusted FCR calculated from data provided in Meloche et al. (2018a). 5. The statistical analysis undertaken by the authors tested for linear and quadratic changes with decreasing feed intake.

8.2 Modifying growth using qualitative manipulation of diet density

It was clear from the first two trials that the quantitative control of feed intake can be used effectively to modify the growth curve of broilers and reduce the incidence and severity of BMM. However, in some regions of the world it may not be possible to quantitatively control feed accurately due to the lack of (accurate) weighing equipment. Another practical approach to modify the growth curve is by reducing dietary nutrient allocation **qualitatively** by manipulation of the amino acid (AA) and energy density of the diet.

Two studies investigated whether reducing the dietary nutrient density could reduce the nutrient intake, modify the growth curve and thereby affect the incidence of BMM (Meloche et al., 2018b, internal Aviagen trial). Previous studies at Aviagen had shown that broilers could adjust their voluntary feed intake to compensate for approximately 5% reduction in dietary nutrient density but in both studies here, contrary to expectations, the broilers of current genotypes were able to compensate for a 10% reduction in energy by eating more feed and still achieving the same final body weight **(Table 4)**. As a consequence the broiler growth curve was not successfully modified and there were very little differences in BMM among the different treatments.

There was no evidence that reducing diet nutrient density reduced the incidence of either WB or WS this was consistent across the two experiments **(Table 4)**. Indeed reducing diet nutrient density between 8 – 14d and 8 – 25d (Meloche et al., 2018b, Experiment 1) and 0 – 11d and 0 – 28d (internal Aviagen trial) increased the incidence of WB and WS, supporting the observation that poor early nutrition may increase the risk of BMM **(see Section 10.2)**. It was concluded that reducing diet nutrient density was not an effective method of reducing BMM.

Table 4. Summary of feed density trials[1,2].

Study	Experiment	Diet Density	Processing Age (d)	Severe WB %	Severe WS %	BW kg (lbs)	FCR	BMY (%)
Meloche et al. (2018b)[3]	**1**	**100, 100, 100** **95, 100, 100** **95, 95, 100** **95, 95, 95** **90, 100, 100** **90, 90, 100** **90, 90, 90**	**35**	**18.2^{bc}** **32.9^{a}** **30.4^{ab}** **18.6^{bc}** **34.7^{a}** **24.8^{abc}** **26.8^{abc}**	**31.6** **41.3** **31.7** **28.8** **41.2** **26.2** **34.5**	**3.162 (6.97)** **3.183 (7.02)** **3.171 (6.99)** **3.124 (6.89)** **3.140 (6.92)** **3.176 (7.00)** **3.132 (6.90)**	**1.582^{b}** **1.586^{b}** **1.605^{b}** **1.646^{ab}** **1.582^{b}** **1.622^{b}** **1.698^{a}**	**23.72^{b}** **23.91^{ab}** **23.96^{ab}** **23.99^{ab}** **23.89^{ab}** **24.14^{ab}** **24.34^{a}**
	2	**100, 100, 100, 100** **95, 100, 100, 100** **95, 95, 100, 100** **95, 95, 95, 95** **90, 100, 100, 100** **90, 90, 100, 100** **90, 90, 90, 90**	**43**	**36.5^{a}** **26.1^{ab}** **37.7^{a}** **39.3^{a}** **29.9^{ab}** **38.9^{a}** **20.8^{b}**	**64.5^{a}** **55.9^{ab}** **59.0^{ab}** **62.0^{a}** **45.3^{ab}** **50.3^{ab}** **42.3^{b}**	**3.792 (8.36)** **3.827 (8.44)** **3.766 (8.30)** **3.777 (8.33)** **3.772 (8.32)** **3.798 (8.37)** **3.789 (8.35)**	**1.675^{c}** **1.684^{c}** **1.715^{bc}** **1.749^{ab}** **1.701^{bc}** **1.730^{bc}** **1.806^{a}**	**26.43^{b}** **26.65^{b}** **26.39^{b}** **26.35^{b}** **27.36^{a}** **26.46^{b}** **26.58^{b}**
				WB score[5]	**WS score[5]**			
Internal Aviagen Trial[4]		**100, 100, 100, 100,** **100, 90, 100, 100, 100,** **100, 90, 90, 100, 100,** **100, 90, 90, 90, 100,** **100, 90, 90, 90, 90,** **100, 100, 90, 100, 100,** **100, 100, 90, 90, 100,** **100, 100, 90, 90, 90,** **100, 100, 100, 90, 100,** **100, 100, 100, 90, 90,** **100, 100, 100, 100, 90,** **100, 90, 90, 90, 90, 90**	**62**	**0.55^{de}** **0.85^{abc}** **1.04^{a}** **0.63^{cde}** **0.68^{cde}** **0.96^{ab}** **0.69^{cde}** **0.75^{bcde}** **0.53^{e}** **0.77^{bcd}** **0.57^{de}** **0.94^{ab}**	**0.89^{ef}** **1.21^{abc}** **1.37^{a}** **0.77^{f}** **0.95^{ef}** **1.19^{abcd}** **0.93^{ef}** **0.98^{cdef}** **1.06^{bcde}** **0.97^{def}** **0.97^{def}** **1.24^{ab}**	**3.960 (8.73)** **4.157 (9.16)** **4.191 (9.24)** **4.124 (9.09)** **4.181 (9.22)** **4.139 (9.12)** **3.993 (8.80)** **4.122 (9.09)** **3.950 ((8.71)** **4.076 (8.99)** **4.223 (9.31)** **4.339 (9.57)**	**2.112^{c}** **2.093^{c}** **2.116^{c}** **2.197^{b}** **2.232^{ab}** **2.136^{c}** **2.217^{ab}** **2.260^{a}** **2.200^{b}** **2.229^{ab}** **2.115^{c}** **2.260^{a}**	

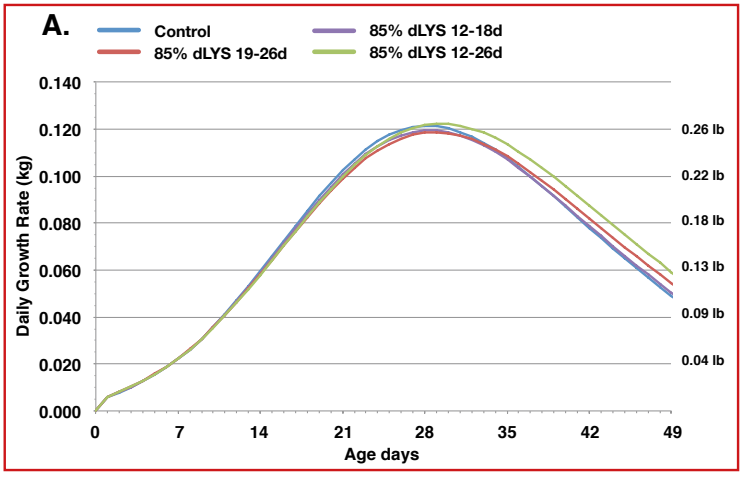
1. Birds were fed diets either as Aviagen recommendation (100) or all nutrients were reduced to either 95 or 90% of Aviagen recommendations.
2. Results within a box with the same superscript are not significantly different at $p<0.05$. Where no superscript is shown there were no significant differences
3. Feeding periods were 8-14, 15-25, 26-42 and 43-48d
4. Feeding periods were 0-11, 12-28, 29-40, 41-48 and 49-62d.
5. Breast fillets were scored for the presence of myopathy as either 0 (none), 1 (mild), 2 (moderate) or 3 (severe). Myopathy score is the average score for all the fillets evaluated.

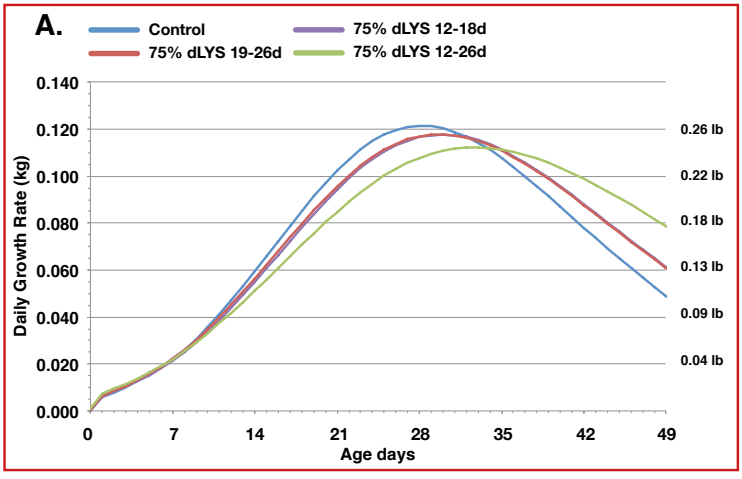
8.3 Lysine deletion

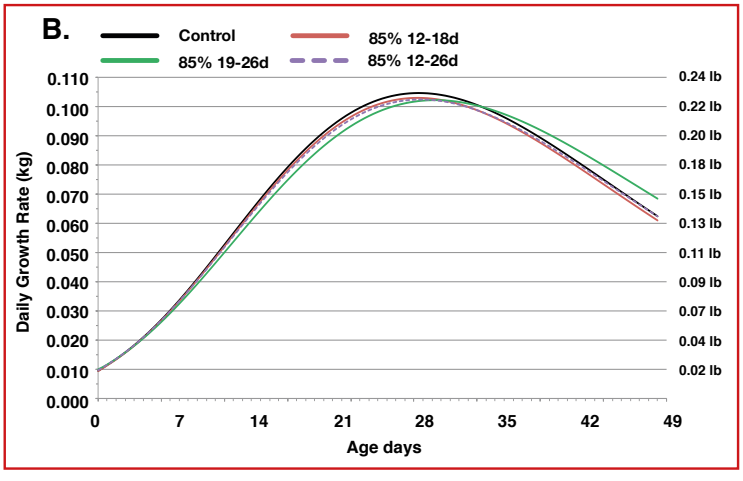
In order to be most effective, a qualitative approach to feed control must not result in the broiler increasing voluntary feed intake to compensate for the change in dietary nutrient density. One possible approach is to reduce the dietary concentration of a single critical Amino Acids (AA). If an AA is present at high levels in breast muscle, then by definition growth must be reduced when there is an inadequate intake of that AA for maximum growth. It is also important that the AA selected is not essential for feather development, otherwise another potential problem is created due to impaired feathering. Digestible lysine (dLYS) is therefore an ideal candidate as the chicken is unable to recognize a change in dLYS level in the diet and adjust its feed intake. Lysine represents ~ 7% of the amino acid content of feather-free carcass protein but only ~ 2% of the amino acid content of feathers. Furthermore, feed-grade lysine is commonly supplemented in poultry diets and can therefore be easily removed from the diet at the feed plant.

Meloche et al. (2018c, d) showed that reducing only the dietary dLYS levels below Aviagen recommendations could alter the growth curve **(Figure 12)** and significantly reduce the incidence and severity of both WB and WS **(Table 5)**. At early processing ages (41 – 48d) a 75% reduction in dLYS in diets fed between 12 – 26d reduced the incidence of severe WB and WS, although there was also a significant reduction in breast meat yield (Meloche et al., 2018c,d) and reduced body weight and increased FCR (Meloche et al., 2018d). Broilers processed at a later age (61d), with sufficient recovery time, had a significant reduction in WB and WS when an 85% reduction in dLYS was used without significantly affecting broiler performance, which was not observed at the earlier processing ages (Meloche et al., 2018c).

Figure 12. The effect of dLYS deletion on daily growth rate (Meloche et al., 2018c: a. Experiment 1; b. Experiment 2).







To confirm the observations from the research trials Aviagen, in collaboration with a customer, conducted a large scale field trial using 85% dLYS deletion in diets fed between 15 – 32d to male and female broilers that were processed at 58d **(Table 5)**. The incidence of WB and WS was very low in the females and so no difference in incidence was observed. The males had a much higher incidence of severe WB in the control group (11.6%) and the incidence in the dLYS treatment group was significantly reduced to ~ 55% of the control. In both the males and the females the dLYS treatment did not significantly reduce broiler performance or processing yield **(Table 5)**.

Table 5. Summary of lysine deletion trials.

Study	Experiment	Treatment[1]	Procssing Age (d)	Severe[2] WB %	Severe[2] WS %	BW kg (lbs)	FCR	BMY (%)
Meloche et al. (2018c)	1	Control 85% 12 – 18d 85% 19 – 26d 85% 12 – 26d 75% 12 – 18d 75% 19 – 26d 75% 12 – 26d	48	36.6^{a} 26.1^{ab} 37.7^{a} 39.3^{a} 29.9^{ab} 38.9^{a} 20.8^{b}	64.3^{a} 55.9^{ab} 59.2^{ab} 62.0^{a} 45.6^{ab} 50.4^{ab} 42.3^{b}	3.594 (7.92) 3.566 (7.86) 3.585 (7.90) 3.629 (8.00) 3.581 (7.89) 3.606 (7.95) 3.536 (7.80)	1.599 1.600 1.572 1.589 1.579 1.610 1.609	27.3 27.3 27.2 27.4 26.8 27.1 26.3 p = 0.003
	2	Control 85% 12 – 18d 85% 19 – 26d 85% 12 – 26d	61	39.3^{a} 33.0^{ab} 32.4^{ab} 18.8^{b}	38.3^{a} 22.3^{b} 31.5^{ab} 17.8^{b}	4.545 (10.02) 4.483 (9.88) 4.474 (9.86) 4.457 (9.83)	1.954 1.874 1.924 1.848	28.6 28.9 28.6 28.0
Meloche et al. (2018d)	1	Control 75% 15 – 25d	41	58.6^{a} 19.7^{b}		3.028 (6.68)a 2.848 (6.28)b	1.494^{a} 1.532^{b}	25.2^{a} 23.7^{b}
	2	Control 75% 15 – 25d	43	50.0^{a} 21.7^{b}		3.543 (7.81) 3.481 (7.67)	1.590^{a} 1.699^{b}	
Commercial Trial	Females	Control 85% 15 – 32d	58	0.4 0.4	0.9 0.0	3.508 (7.73) 3.576 (7.88)		29.25 29.59
	Males	Control 85% 15 – 32d	58	11.6^{a} 6.4^{b}	3.8 2.6	4.473 (9.86) 4.521 (9.97)		28.72 28.37

1. The percentage shown is the digestible lysine level in the diet as a percentage of the control (Aviagen recommendations) diet.
2. Severe WB and WS are those in the highest score category. Meloche et al. (2018c,d) used a 0, 1 and 2 scoring system and the commercial trial used a 0, 1, 2 and 3 scoring system.

Based on the results of these trials it can be concluded that reductions in dLYS (dLYS deletion) for specific age periods is an effective and practical approach to reduce the incidence and severity of WB and WS. However, this method needs to be implemented properly. In the extreme approaches (25% reduced dLYS fed between 12 and 26 days of age in a 48 day grow-out period and a 15% reduction in dLYS fed between 12 and 40 days of age in a 61 day grow-out period) substantial reductions in WB and WS are achieved but breast meat yield was also reduced. For these extreme treatments the reduction in dLYS was fed for a period that represents approximately 35% (12-26 days in a 48 day grow-out period) and 45% (12-40 days of age in a 61 day grow-out period) of the total feed intake for the respective grow-out periods. Therefore, the treatment periods need to be shorter (i.e. the treatments need to be fed for a lower proportion of the total feed intake of the grow-out period) than those used in the extreme treatments described here.

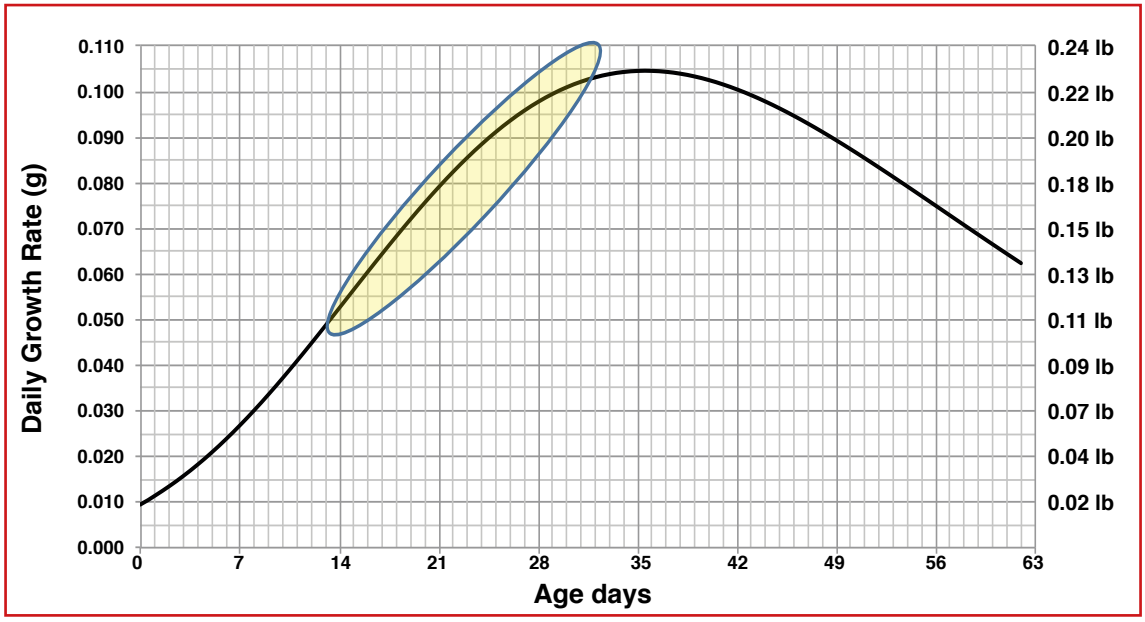
The age at which restriction treatment is started is also an important consideration as satellite cell proliferation must not be impaired. Delaying initiation of the treatment until 14 days of age is recommended as it allows a 'margin of safety' for the aforementioned critical period of satellite cell proliferation. Generally speaking, the ideal age period for utilizing the reduced dLYS approach appears to be between 14-32 days of age **(Figure 13)**. Maximal broiler growth occurs between 28 and 35 days of age and the data collected so far shows no real benefit of applying treatment near

or after the age of maximal growth. When implementing the reduction in dLYS method to reduce incidence and severity of WB and WS the following should be considered:

1. Age of starting dLYS reduction – not before 14 days of age.
2. Length of treatment period – < 25% of total grow-out feed intake (you can utilize Aviagen broiler performance objectives to estimate this value – see example below).
3. The level of dLYS targeted during this period should be 85% of Aviagen broiler nutrition specifications and it is not advised to use dLYS levels < 85%. Importantly, reduce only dLYS and do not change the dietary constraints for any other AA.

Age range to utilize the LYS deletion approach is between 14 to 32 days of age; however, point (2) should be respected to arrive at the appropriate age period of implementation.

Figure 13. The growth rate curve of the broiler showing the optimum period for dLYS deletion.



Example of the calculation of time dLYS reduction should be fed.

Ross 308 A/H processed at 42d:

Cumulative feed at 42d = 4739g / 10.4lbs

25% of 4739g/10.4lbs = 1185g / 2.6lbs

Feed to 14d = 537g / 1.2lbs

Feed intake at which lysine deletion should be stopped = 1185g (2.6lbs) + 537g (1.2lbs) = 1722g (3.8lbs), which is normally achieved by 25 – 26d

8.4 Early Growth and BMM

The internal Aviagen studies where either feed intake **(Table 3)** or nutrient density **(Table 4)** were only reduced in the first 11 days of age showed an increase in the incidence of WB and WS. As noted in **Section 6.2**, reduced nutrient intake during the first week after hatch can reduce satellite cell proliferation which is essential for muscle repair (Velleman et al., 2010). Analysis of commercial field data has also shown that reduced first week growth resulted in an increased incidence of WB. The evidence from both research and field data supports the conclusion that poor growth in the first 7 – 12d after hatch increases the risk of BMM.

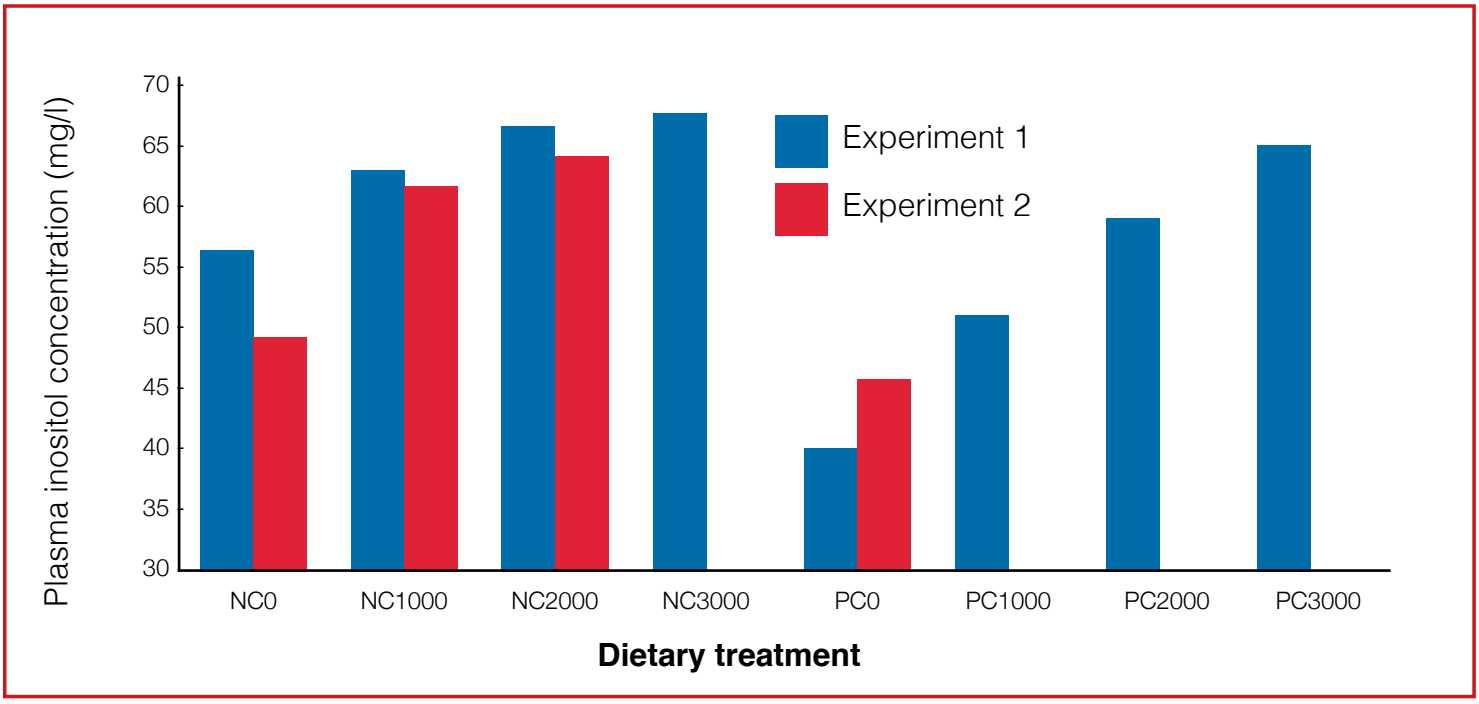
9. Nutrition and BMM

9.1 Phytase

Phytase has been adopted as a common practice by the poultry industry as a way of improving the digestibility of plant-bound phosphorus. Normal inclusion rates for this enzyme range between 500 and 750 phytase units/kg (2.2lbs) finished feed. The addition of this enzyme at doses higher than typically recommended has been shown to improve broiler performance.

Previous studies have found that some phytases have the ability to degrade phytic acid in plant-based ingredients to its simplest form (inositol) if provided at sufficient dose. Plasma inositol has been demonstrated to increase with increasing phytase dose (**Figure 14**, Cowieson et al., 2014). Once this compound is absorbed and re-phosphorylated within the cell it acts as a potent cellular antioxidant, among other functions. Breast muscle tissue affected by myopathies like WB is under significant oxidative stress. Antioxidants delivered to the target tissue should be of benefit under such conditions. Therefore, a series of studies were conducted to evaluate the effects of phytase at recommended or higher levels on incidence and severity of BMM.

Figure 14. Effect of RONOZYME® HiPhos GT (expressed as FYT/kg) on plasma myo-inositol concentrates in broiler chicks fed diets containing insufficient (NC) and sufficient (PC) available P and Ca levels (Cowieson et al., 2014).



A first study conducted at Aviagen's USA trial farm, compared a control treatment with no added phytase to 3 different levels of phytase (500, 1500 and 3000 phytase units/kg (2.2lbs)) in predominantly corn-soya diets. Supplementing phytase at levels above manufacturer recommendations resulted in improved live performance and carcass traits **(Table 6)**. However, supplemental phytase did not have a statistically significant impact on BMM, although numerical trends towards lowering severity were noted. This is an interesting observation considering that treatments showing an ability to increase growth rate and breast meat deposition often result in an increased incidence of myopathies.

Table 6. Male broiler performance and muscle myopathies when fed various levels of added phytase at 49 days of age.

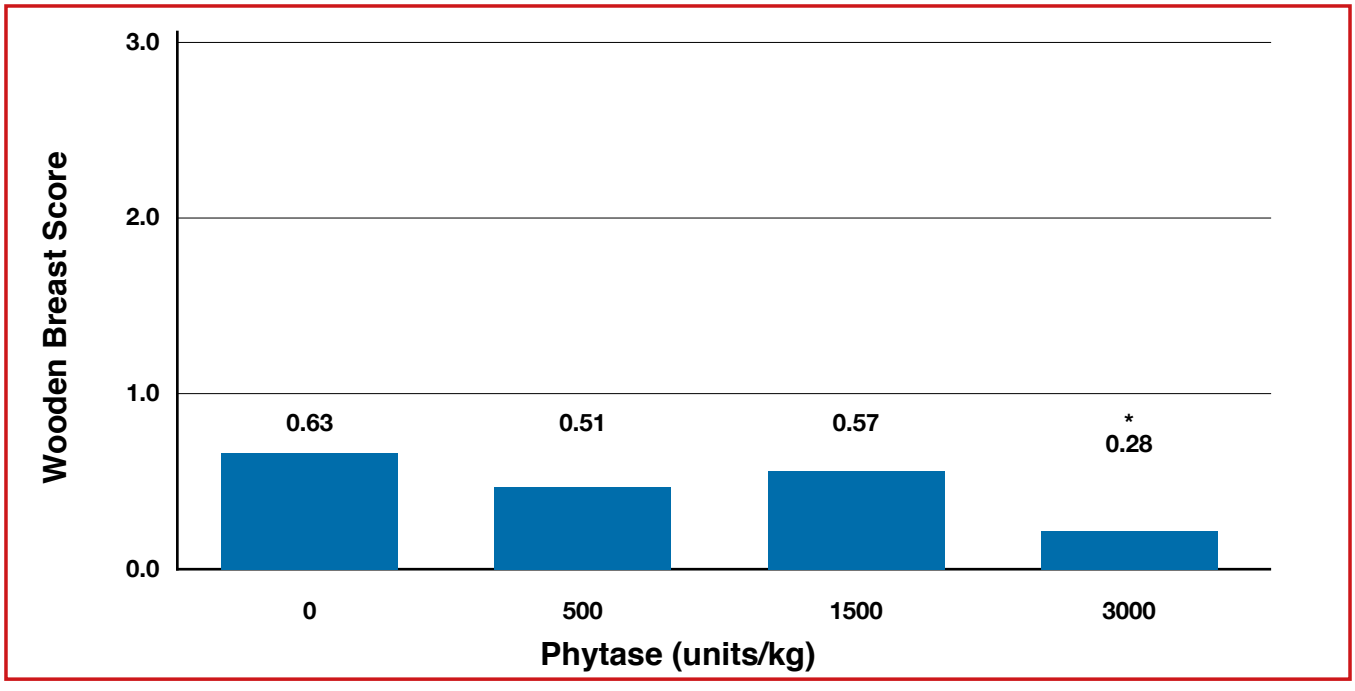
Phytase level	BW kg (lbs)	FCR adj[1]	Carcass Yield[2]	Total white meat yield[2]	Wooden breast score[3]	White striping score[3]
Control (0 FTU)	3.47 (7.65)	1.69[a]	77.89[b]	28.07	1.17	1.05
500 FTU[4]	3.50 (7.72)	1.67[ab]	78.33[a]	28.28	0.99	0.93
1500 FTU	3.54 (7.80)	1.67[ab]	78.28[a]	28.12	1.01	1.01
3000 FTU	3.57 (7.87)	1.65[b]	78.37[a]	28.24	1.06	0.99
P Values	0.08	0.04	0.04	0.83	0.32	0.24

1. Feed conversion adjusted for mortality and BW
2. Expressed relative to the live weight
3. Myopathy average score (ranging from 0 to 3) where 0 is unaffected and 3 is severely affected
4. Phytase units

A follow up study conducted at Aviagen's UK trial farm, using a similar treatment design but with predominantly wheat-soya diets resulted in live and carcass performance improvements as phytase was added to the feed above recommended levels, in parallel with responses reported in the first study. As observed in the first study, increases in performance did not translate into an increased incidence of BMM. Furthermore, supplementing 3000 phytase units/kg significantly reduced WB score in Ross 708 broilers at 46 days of age **(Figure 15)**.

The findings in this second study conducted by Aviagen are in agreement with those from York et al. (2016) who described a reduction in WB severity score when feed was supplemented with an E. coli derived phytase at three times the recommended dose from the manufacturer. However, York et al. (2016) also added an antioxidant and organic minerals to the feed, so it is not possible to conclude that the reduction in WB severity was an effect derived solely from phytase super doses. Interestingly, the authors also reported an increase in breast meat yield and live performance parameters without deleterious effects on BMM, in agreement with all phytase studies conducted at Aviagen. It is therefore plausible that super doses of phytase (>750 FTU) may support optimal broiler performance without increasing the risk of BMM.

Figure 15. Wooden breast average score in Ross 708 male broilers at 46 days of age fed various levels of phytase supplementation.



9.2 Lysine deletion and phytase super-dosing

Given the results obtained from the various lysine deletion **(see Section 8)** and phytase super-dosing studies, a study was conducted at the Aviagen UK trial farm, to evaluate possible synergy between lysine deletion and phytase super-dosing strategies. Various lysine deletion periods (11-21d; 11-29d; 15-25d; 15-33d; 19-25d; 19-39d) and phytase doses (500 or 1500 phytase units) were fed, and broiler performance and muscle myopathies assessed at 38d and 48d. There were no observed synergistic effects reported in this study, although myopathies were consistently lowered with a lysine deletion approach regardless of phytase level.

9.3 Arginine

Arginine (ARG) is typically not limiting in corn-based commercial broiler feeds, but under certain circumstances it can be when other cereals are used as the primary grain source. This AA has known roles in synthesis of nitric oxide and muscle creatine, which are compounds that have, for example, wound healing properties, vascular flow and immune functions. A preliminary study conducted at the Aviagen UK trial farm evaluated current Aviagen recommendations for ARG:LYS ratio (107) versus a higher ratio (120). Responses to myopathies were variable, and as a result the study was repeated. Results from the second study suggested that feeding a ratio of 120 throughout grow-out improved live performance and carcass traits relative to current Aviagen ARG:LYS recommendations. A slight benefit in lowering the incidence of SS when feeding a 120 ratio was observed early in grow-out (32d), but this response was inconsistent later in life (39d or 46d). Inconsistent results were observed with the feeding of a higher ratio when monitoring the incidence of WB. Overall results suggest that higher levels of ARG could provide some benefit in lowering muscle myopathies, but the effects are not observed consistently – suggesting the response is likely multifactorial.

9.4 Histidine

Histidine (HIS), in combination with alanine, forms the dipeptide carnosine which is highly concentrated in chicken breast muscle cells and has known antioxidant properties. This dipeptide is not available in feed grade form. Amounts of alanine in feeds are typically sufficient especially because this AA can be synthetized from other AA; however, HIS is an essential AA and must be supplied in the diet. A study was conducted to evaluated the HIS:LYS ratio at the UK trial farm. A normal ratio of HIS:LYS ratio (40) commonly seen in commercial feeds was compared to a much higher ratio (70), to evaluate any potential impact on muscle myopathies. A higher ratio resulted in broilers with body weight and FCR improvements although these responses were inconsistent at different ages. No effects on BMM were observed with the different HIS:LYS ratios.

9.5 Organic trace minerals

Due to the higher bioavailability of organic trace minerals, some suppliers of these micro-nutrients have argued that their inclusion in commercial feeds could assist in lowering BMM (e.g. zinc involvement in wound healing). Therefore, a study was conducted in the UK, where 100% of Aviagen trace mineral recommendations for copper, zinc, manganese, and selenium were fed as organic sources. In general, productive responses from feeding organic sources of copper, zinc, manganese and selenium were similar to broilers fed only inorganic forms of these trace minerals. A slight improvement in eviscerated yield was observed at 39d and 46d when feeding organic sources, but overall there was no indication that these more bioavailable forms of minerals led to a decrease in BMM.

9.6 Anticoccidial programs

There are three types of coccidiosis control programs: vaccines, ionophores, or chemicals or combinations of the three types. It has been recognized that differing anticoccidial programs can significantly impact growth rate and overall performance of broilers. Dalle Zotte et al. (2015) reported that birds fed certain anticoccidial programs had a higher incidence of severe white striping than those birds given no anticoccidial additive or vaccinated. A study was conducted at the Aviagen USA trial farm to evaluate the effects of various anticoccidial programs on incidence of BMM. Birds were administered one of various possible anticoccidial treatments: vaccine; chemical; three different ionophores; a chemical/ionophore combination; or a chemical followed in the next feed phase by an ionophore at different ages. Results from the study are presented in **Table 7.**

Table 7. Live performance and incidence of myopathies in broilers provided various anticoccidial programs at 62 days of age.

Amino acid density	BW kg (lbs)	FCR adj[1]	Carcass Yield[2]	Wooden breast score[3]	White striping score[3]
Vaccine	**4.69 (10.34)^{ab}**	**1.87^{c}**	**77.85^{bc}**	**0.76^{c}**	**1.06**
Ionophore A	**4.61 (10.17)^{abc}**	**1.90^{c}**	**78.16^{abc}**	**1.19^{ab}**	**1.25**
Ionophore B	**4.72 (10.41)^{ab}**	**1.87^{c}**	**78.24^{ab}**	**1.10^{b}**	**1.19**
Ionophore C	**4.74 (10.45)^{a}**	**1.87^{c}**	**77.79^{c}**	**1.14^{b}**	**1.16**
Chemical	**4.37 (10.43)^{d}**	**2.05^{a}**	**78.53^{a}**	**1.30^{ab}**	**1.21**
Chemical/Ionophore	**4.48 (9.88)^{cd}**	**2.00^{ab}**	**78.27^{ab}**	**0.97^{bc}**	**1.11**
Chem-11d-Ionophore[4]	**4.53 (9.97)^{cd}**	**1.90^{c}**	**78.41^{a}**	**1.21^{ab}**	**1.22**
Chem-25d-Ionophore[4]	**4.63 (10.21)^{abc}**	**1.87^{c}**	**78.34^{a}**	**1.16^{b}**	**1.12**
Chem-39d-Ionophore[4]	**4.57 (10.08)^{bc}**	**1.91^{bc}**	**77.75^{c}**	**1.50^{a}**	**1.33**
P Value	**0.009**	**0.0001**	**0.0001**	**0.005**	**0.39**

1. Feed conversion adjusted for mortality and BW.
2. Expressed relative to the live weight.
3. Myopathy average score (ranging from 0 to 3) where 0 is unaffected and 3 is severely affected.
4. A chemical was supplemented to the feed at either 0-11 days, 0-25 days, or 0-39 days. All these various feeding periods were immediately followed by an ionophore in the feed until 62 days of age.

The different anticoccidial programs were tested in an environment of low coccidiosis challenge to better evaluate the specific effects on growth rate, FCR and carcass component yield. Although no impact was reported for WS, the different anticoccidial programs dramatically influenced incidence of WB. Broilers administered with the vaccine had significantly lower incidence of WB, whereas the chemical and the chemical/ionophore programs were the highest. The growth curve trajectory of birds given the vaccine was moderated after 15d compared to those fed the ionophore treatments. It is hypothesized that the WB effects are attributable to differences in growth curve trajectories. The well-known negative interaction of the chemical with high environmental temperature and bird thermo-regulation appears to have impact as well since this treatment had a higher incidence of WB than birds given vaccine, despite having a much lower growth rate.

A follow up study using a similar treatment layout to the previous one was conducted at the Aviagen USA Trial farm, again with the additional treatment of a “bio-shuttle program” being included. The latter treatment consisted of a combination of coccidiosis vaccine at hatch and an ionophore fed only during the second feed. The results from this study did not replicate the broiler performance or BMM effects observed in the first study. Unlike results from the first trial, there was very little growth curve moderation after 15d in the vaccine treatment. Consequently, the significant myopathy effects reported

in the first trial were not replicated. It appears that coccidiosis vaccination can potentially reduce the incidence and severity of WB and WS if the growth curve trajectory is sufficiently moderated during the mid-life period (e.g. 15-32 days of age).

9.7 Pre-starter feed

The importance of adequate feeding during the brooding period, where maximum satellite cell proliferation occurs, has been documented in the literature (Harthan et al., 2013; Mann et al., 2011; Velleman et al., 2010, 2014; Powell et al., 2014) and demonstrated in a previously described Aviagen study where feed intake control was applied only during the first 11 days of life. The latter trial revealed how BMM worsened when broilers were control-fed to 95% of ad libitum during their first feeding phase. Therefore, a study was conducted at the Aviagen USA trial farm where birds were fed either a pre-starter feed (0-11 days of pre-starter; 11-14 days of starter) or a regular starter feed program (0-14d) meeting all Aviagen nutrient recommendations (2014). The pre-starter feed was fortified with higher levels of AA, vitamins C and E, and used food-grade soybean oil instead of poultry fat. The pre-starter fed birds had improved 14d body weight and better livability throughout the life of the flock. No other performance benefits were reported across the different time points. Regarding the incidence of BMM, improvements to WS were observed at 42d but not 56d, and no improvements to WB were seen. There appears to be live performance benefits when using a pre-starter feed but the impact on BMM requires further investigation. Clearly, the definition of a "pre-starter" in terms of specific nutrient profile can be highly variable and this must be taken into consideration when trying to generalize the effects of a pre-starter diet.

9.8 Antioxidants

A possible factor impacting BMM is oxidative stress. While Kuttappan et al. (2012e) showed that supplementing diets using good fat quality with vitamin E up to 400 IU/kg (2.2lbs) did not have any impact on WS, if free radical production overwhelms the body's ability to neutralize them, oxidative damage can occur in cell membranes and trigger a cascade of reactions which ultimately hinders integrity of body tissues.

For that reason, soy oil was purposely oxidized to a predetermined level of peroxides and then used to manufacture broiler feed in an Aviagen study. This oxidized soy oil contained a known level of peroxides (225 mEq/kg (2.2lbs)) and was fed to broilers at 3% inclusion. Addition of the antioxidant ethoxyquin (125 ppm), heat-stable vitamin C (200 ppm), vitamin E (180 IU) or combinations were evaluated for their impact on BMM. **Table 8** depicts how the addition of any of the antioxidants, or combination, lowered WB at 49d, although this effect was not replicated at 62d. Statistical analysis showed that at 49 days all antioxidants significantly reduced severe (score 3) WB by 38-48% **(Table 9)** and these results were supported by these antioxidants significantly reducing plasma LDH compared to control. No beneficial effect on WS was detected with the addition of antioxidants.

Table 8. Live performance and incidence of myopathies in broilers supplemented with different antioxidants at 49 days, when provided feed with highly oxidized fat.

Antioxidant	BW kg (lbs)	FCR adj[1]	Wooden breast score[2]	% of WB scores 2&3[3]	White striping score[2]
No antioxidant	3.59 (7.91)	1.71	1.19[a]	37	1.28
Ethoxyquin	3.57 (7.87)	1.70	1.09[ab]	32	1.18
Vitamin C & E	3.63 (8.00)	1.68	0.86[b]	27	1.16
Ethoxyquin + Vitamin C & E	3.61 (7.96)	1.67	1.10[ab]	33	1.33
P Value	0.33	0.06	0.04	0.37	0.39

1 Feed conversion adjusted for mortality and body weight.
2 Myopathy average score (ranging from 0 to 3) where 0 was unaffected and 3 was severely affected.
3 Wooden breast scores 2 and 3, expressed as a percentage relative to all the breast fillets for that treatment group.

Table 9. Effect of different antioxidants on the incidence of severe wooden breast (score 3) in Ross 708 broilers at 49 days of age.

Antioxidant	WB score 3 (% of total)	Relative change vs oxidized oil with no antioxidant	P Value vs oxidized oil with no antioxidant
No antioxidant	29.0	-	-
Ethoxyquin	15.0	-48%	0.017
Vitamin C & E	17.0	-41%	0.044
Ethoxyquin + Vitamin C & E	18.0	-38%	0.065

If birds receive feed with oxidized fat/oil, they are more susceptible to oxidative stress and would benefit from the addition of effective antioxidants.

9.9 Guanidinoacetic acid

Guanidinoacetic acid is commercially available in most countries, and is a metabolic precursor for creatine. The compound creatine is formed in protein metabolism, and is involved in cellular energy supply for muscle contraction. In diets that only contain plant-based ingredients it is possible that there is insufficient creatine available.

Recent work from North Carolina State University by Cordova-Noboa et al. (2018) indicated that the addition of this compound may help lower the incidence and severity of WB. Therefore, a study was conducted at the Aviagen USA trial farm where this compound was added to broiler feeds (600g (1.3lbs)/MT) and compared with a control feed. There were some slight improvements observed in WB (at 49d but not at 56d) when guanidinoacetic acid was supplemented in the broiler feed. No effects on WS at either age were noted. Interestingly, an increase in breast meat yield occurred with the addition of this compound, and the improvement in yield did not translate into increased WB. This effect is similar to superdosing phytase in some of our previously described studies. In summary, this compound appears to enhance productivity of broilers without exacerbating BMM.

Aviagen undertook two field trials in Europe under commercial conditions to evaluate the effects of adding guanidinoacetic acid to plant-based diets on broiler performance and BMM. In both trials, two farms were used, each with two houses that were fed either a standard diet or diet supplemented with 600g (1.3lbs)/tonne guanidinoacetic acid. In both trials the houses fed the guanidinoacetic acid had significantly lower incidence of WB and WS ranging from a 17 – 31% reduction in incidence. In three of the four houses fed guanidinoacetic acid there was also an improved growth performance compared to the control feed houses which is consistent with previous observations.

9.10 Other Products

A commercially marketed product containing betaine, ascorbic acid and unidentified phytogenic compound has been claimed to affect the incidence of BMM. It received considerable interest from poultry companies due to their initial claims that the product could significantly lower the incidence and severity of WB. Reported testing in Europe and Brazil led to this product being advertised as having beneficial properties against WB.
To test the product's efficacy against BMM, a study was conducted at the Aviagen USA Trial farm using the manufacturer's recommendations, and in a separate treatment including the product for

an extended dosing period. In **Table 10**, the results from the study are summarized, and it was concluded that no effects on WB or WS, or any productive parameter measured were observed when this product was supplemented at any of the doses tested.

Table 10. Live performance and incidence of myopathies in broilers supplemented with the betaine, ascorbic acid and unidentified phytogenic compound product at 49 days of age.

	BW kg (lbs)	FCR adj	Wooden breast score[3]	% of WB scores 2&3[4]	White striping score[3]
Control	**3.76 (8.29)**	**1.58**	**1.69**	**50.5**	**1.72**
Product[1]	**3.73 (8.22)**	**1.60**	**1.66**	**50.7**	**1.64**
Product Extended[2]	**3.72 (8.20**	**1.60**	**1.66**	**53.5**	**1.83**

1. Treatment followed the manufacturer's inclusion recommendations: 0.1% in the Starter, 0.065% in the Grower, and 0.035% in the Finisher feed.
2. Treatment was added at 0.1% in the Starter, 0.065% in the Grower, and 0.035% in all three remaining feed until slaughter.
3. Myopathy average score (ranging from 0 to 3) where 0 was unaffected and 3 was severely affected.
4. Wooden breast scores 2 and 3, expressed as a percentage relative to all the breast fillets for that treatment group.

10. Management and BMM

10.1. Incubation

The chick embryo develops in well-defined stages over 21d of incubation. Extremes of incubation conditions can affect the rate and relative development of tissue occurring, without necessarily being fatal. For example, there is plentiful evidence that high or low egg shell temperature during incubation will affect embryonic growth rate, while high levels of CO_2 in the earlier stages of incubation can affect capillary branching in the chorio-allantoic membrane (Verholst et al., 2011).

Skeletal myofiber hyperplasia development takes place both before and immediately after hatch, and suboptimal conditions from around day 14 of incubation may affect growth and breast meat development post-hatch. For this reason the advice given by Aviagen in the past has been to avoid overheating the embryo in the final stages of incubation. However, there are some less common incubation techniques which affect the growth of breast muscle post-hatch, as well as breast meat percentage at slaughter and BMM, including:-

- short periods of high temperature alternated with lower temperatures (Pietsun et al., 2008)
- exposure to green light during incubation (Rozenboim et al., 2004)

Reports in the scientific literature often describe results of experiments performed with small numbers of eggs incubated in specialist small scale incubators. Recent attempts at the Aviagen USA trial farm to scale these schemes up in larger commercial-style single stage incubators have not given clear results, presumably because it is difficult for even a small commercial incubator to deliver identical light and heat exposure at every point in the machine.

All of the small scale trials reported have focused solely on the potential benefits from increased satellite cell number delivering more breast meat at slaughter. While it is assumed that the extra

satellite cells may also be available for healing damage to the breast muscle, this has not been explicitly tested. Again, in Aviagen's larger-scale incubation trials of thermal manipulation or light during incubation, their impact on WB or any of the other BMM has been limited, although neither did any harm to hatchability or breast muscle growth or quality.

For now, the best incubation advice to minimize BMM is to control incubation conditions and avoid over-heating in the later stages of incubation. Thermal manipulation or lighting on a large scale would need careful monitoring to ensure implementation is as intended and there are no unintended consequences. Further research is continuing in this area.

10.2. Access to feed after hatch

Aviagen has for many years advised producers to feed broilers as soon as possible after hatch. This reflected research reported by Noy and Sklan (1997) showing that growth and efficiency were both improved by early feeding because feed in the gut accelerated mobilization and utilization of residual yolk, allowing chicks to dedicate the extra nutrients to growth rather than survival post hatch. In 2000 Halevy et al. showed that feed intake control of chicks during the first week post hatch decreased satellite cell proliferation and muscle growth, with the effect being most prominent if the chicks were left without food or water in the 48 hours immediately post hatch. Velleman et al. (2014) showed similar time limited effects when reducing feed intake by 20% during the first or second week post hatch.

In this case, and unlike the incubation trials, larger scale trials conducted at the Aviagen USA trial farm showed that poor post hatch growth was strongly associated with increased myopathies. Again, the advice given is to ensure that broiler chicks are placed as soon as possible post hatch, achieve good crop fill in the first 24 hours and target 7 day weights as a minimum of 4 times placement weight remains best practice to limit the occurrence of BMM.

10.3. Environmental/bird body temperature

High environmental temperature can result in muscle damage in broilers. Sandercock et al. (2006) showed heat stressed birds had higher levels of plasma creatine kinase, an indicator of muscle damage. Zahoor et al. (2016) tested the hypothesis that BMM were due to an inability of modern broilers to lose heat. They compared a standard temperature profile with a cool profile after 21d of age. No difference in muscle histology or creatine kinase activity was found, although the muscle from the cool treatment had greater stiffness but no difference in shear strength. Similarly an internal Aviagen trial completed at the trial farm in The Netherlands compared growing broilers at a constant 25°C (77°F) after 12d compared to the normal temperature profile declining to 19°C (66°F) but found no difference in the incidence of any BMM.

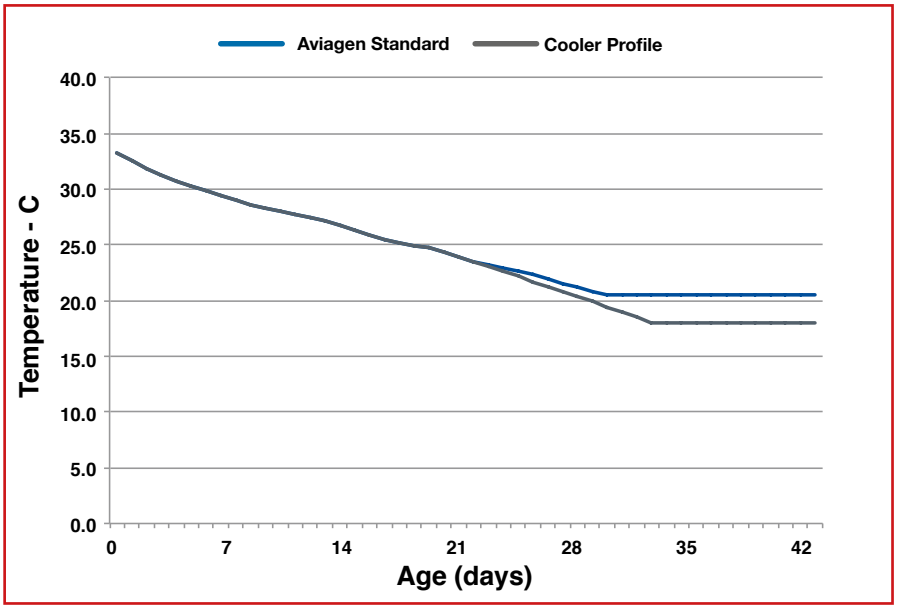
Heat stress during transport to the processing plant has been shown to increase the incidence of PSE in broilers (Holm and Fletcher, 1997; Simões et al., 2009) whereas temperatures below 0°C (32°F) increased the incidence of DFD (Dadgar et al., 2012).

While there is currently no clear published evidence, some field evidence indicates that ensuring broilers are not heat stressed can reduce the incidence of WB, WS or SS, with some poultry producers reporting that incidence of WB decreases when house temperatures are lower. Many who have taken steps to reduce high temperatures at bird level have seen a benefit in a reduction of BMM.

Where BMM are a problem, Aviagen recommends reducing the house temperature and increasing ventilation at bird level to ensure good air movement around birds during the mid and late growth

phases. A suggested temperature profile to reduce the incidence of BMM is shown in **Figure 16**. As some coccidiostats are known to raise body temperature **(Section 9.6)**, their use should be avoided when possible when house temperatures are high.

Figure 16. Suggested cooler temperature profile to use to reduce BMM compared to the standard Aviagen profile. Note that at higher ambient humidities, the temperature may need to be lowered further.



10.4. Carbon-dioxide levels

In poultry houses, carbon dioxide (CO_2) levels will vary with the degree of ventilation and can be regarded as a measure of ventilation levels. Typically during the brooding phase the house will operate on minimum ventilation with sufficient ventilation to maintain CO_2 below 3,000 ppm. However, in many situations the CO_2 levels during brooding can be much higher, for example when outside temperatures are low and the farmer reduces ventilation to save on heating costs: levels in excess of 6,000 ppm have been recorded. As the birds age, they start to generate more metabolic heat and ventilation must be increased to remove this heat. Under these circumstances, CO_2 levels will normally be much lower than 3,000 ppm. High levels of CO_2 in the poultry house may also indicate high levels of ammonia and lower levels of oxygen and the effects of each of these gases cannot be easily separated.

There are two hypotheses which may explain why high levels of CO_2, or conversely low levels of oxygen, might have a negative effect on the incidence BMM. Firstly, high CO_2 during the brooding period may have a negative effect on first week growth: as noted in **Section 8.1**, poor early growth increases the risk of BMM probably due to reduced satellite cell proliferation.

The second possible route is that high levels of CO_2 may induce ascites which in turn would reduce blood circulation in the older bird and thereby increasing the risk of muscle cell damage. An analysis of broiler flock data from one processing plant did observe an increase in BMM in flocks with a higher incidence of ascites. Furthermore, data from Aviagen's selection program have shown that birds with a higher level of blood oxygen saturation have a lower risk of WB.

There is no published data that shows an effect of CO_2 levels during brooding on early growth, indeed the few studies that have investigated CO_2 levels in the broiler house up to 9,000 ppm found no effect

on growth (McGovern et al., 2001; Olanrewaju et al., 2008). A limited analysis of field data from a European broiler producer by Aviagen revealed decreased growth when CO_2 levels in the house exceeded 3,000 ppm, but more data are required to confirm this observation.

Studies have shown that high levels of CO_2 during brooding can increase ascites:

- McGovern et al. (2001) showed higher late mortality due to ascites when brooded with CO_2 of 6,000 versus 600 ppm
- Olanrewaju et al. (2008) observed a linear increase in late broiler mortality and increasing 42d heart weights as CO_2 levels during brooding increased above 3,000 to 6,000 and 9,000 ppm.

There are no published studies showing an increase in BMM due to elevated CO_2 levels. However, given that it is good management practice to maintain CO_2 levels below 3,000 ppm, it is recommended that sufficient ventilation is provided at all times to achieve this.

10.5. Lighting program

The effect of lighting on BMM has not been studied in the published scientific literature, but several studies have shown that light period, intensity and wavelength can all affect breast muscle development.

The effect of light wavelength on breast muscle development was studied by Rozenboim et al. (1999) who showed that birds grown under green light had higher breast meat yield compared to white, blue and red light. More interestingly, this group also showed that birds grown under blue and green light to 5d had more satellite cells per gram of breast muscle (Halevy et al., 1998). Given the importance of satellite cells for muscle repair, the possibility that blue and green light might increase the number of satellite cells in the muscle is being further investigated by the University of Saskatchewan.

Published studies have not shown any direct effect of lighting programs on BMM, but the evidence does suggest that light can affect muscle development. Two internal Aviagen studies did look at the effect of light program on the incidence of BMM and the results suggested that modified lighting programs could be used to reduce the incidence of the WB: the mechanism may be by reducing growth in a similar way to the lysine deletion studies. However, the optimum lighting program to reduce BMM is not yet properly understood and further work is required before any recommendations can be made.

10.6. Litter depth

It has been suggested that some BMM may be triggered by poor blood supply to the breast muscle causing localized muscle cell death. When broilers are sitting on the litter for extended periods of time, the pressure on the breast may cause constriction of the blood supply (ischaemia) to the breast and increase the risk of muscle cell damage. It has been suggested that hard, compact litter may increase the risk of WB and a study at the University of Helsinki (Puolanne et al., 2015 unpublished report) showed that providing soft bedding (exercise mats) reduced the incidence of WB compared to using wood shavings.

It is not suggested that exercise mats be used as a bedding material, but the depth of litter may alter the pressure placed upon the breast when birds sit and potentially help to reduce the incidence of WB. This has not been tested commercially.

10.7. Bird flapping / activity

It has long been known that deep pectoral myopathy (DPM) can be induced by vigorous wing flapping in both broilers and turkeys (e.g. Lien, 2012). Wing flapping results in the breast muscles contracting and the deep pectoral muscles becoming filled with blood. Due to the inelastic sheath around the muscle the pressure within the muscle increases which reduces blood flow in and out of the muscle; this can then result in muscle cell death in the deep pectoral muscles. Vigorous flapping must occur sometime prior to slaughter to result in DPM.

Wing flapping and struggling during shackling in the processing plant has been shown to affect muscle glycogen and lactic acid content at death, which increases the risk of PSE myopathy (Berri et al., 2005; Debut et al., 2003).

To date there is no evidence that BMM can be induced by excessive bird activity, but it should be considered good management practice to reduce excessive wing flapping activity in broilers at all times. Ensuring birds are used to human activity within the house and always handled in a calm and careful way reduces risk of vigorous wing flapping and potential damage to breast muscle.

10.8. Thinning

Many companies practice thinning, that is they remove a percentage of the birds from the broiler house at an earlier age and lower weight than the final depletion age. The thinned birds are used to meet the processing plants requirement for lower bird weights for particular market requirements. Birds remaining in the broiler house until final depletion have the advantage of extra floor, feeder and drinker space which allows enhanced growth until final depletion.

The practice of thinning has two potential routes to affect incidence of BMM. Firstly, activity in the house when the thinned birds are being loaded may disturb the birds remaining and lead to excessive activity and flapping: it is important to ensure that the thinned birds are caught in a quiet, calm manner so that remaining birds are not disturbed. Secondly, extra floor, feeder and drinker space suddenly becoming available for the remaining birds can result in sharp increase in growth rate. Experience in the field has shown that birds that grow faster than 120 g/d post thinning have a higher risk of WB and WS. It is good practice to gradually allow the remaining birds access to the extra available space in the house post thinning.

11. Disease and BMM

There are no clinical signs of BMM on the farm and no evidence of their presence in live birds except for WB where a hardness of the breast can be detected by palpation. In addition, the epidemiology does not support an infectious cause as there is no pattern of spread between houses, farms or complexes which would indicate an infectious agent is involved.

Clearly, BMM can be observed in all breeds/strains of chickens as early as 2 weeks of age with varying prevalence under a wide-range of slaughter weights, management, feeding and rearing systems (Radaelli et al., 2016). WB was detected in different strains around the same time in different continents and this is not how an infectious disease would be expected to behave. There is also good evidence of a metabolic aetiology **(Sections 7.1 and 7.2)**. Bilgili (2016) concluded that there is no evidence of infectious and/or pathogenic agents associated with BMM.

However, some have still questioned whether there could be a role for an infectious agent in WB because of the lymphocytic vasculitis observed by histopathologists in birds with this condition.

While the perivascular infiltrate may be indicative of a lymphocytic phlebitis others have explained the presence of these lymphocytes as a natural immune response. “The lymphoid accumulation around vessels in WB is entirely consistent with and explicable in terms of it being part of the inflammatory response to significant tissue damage (i.e. muscle) when the circulation remains relatively functional and vessels remain patent.” (Personal communication: Alisdair Wood – specialist in poultry histopathology UK). A recent study by Barnes et al. (2017) detected an infectious coronavirus in a trial facility at North Carolina State University, however this finding was not replicated in a more recent study in the UK (unpublished data).

12. Processing

12.1. Effects of bird handling prior to processing

Pre-slaughter flock management and husbandry systems are very important as they relate to growth rate (muscle tissue), bird activity (skeletal and cardiovascular) and consequently in the occurrence of BMM. DPM has been directly associated with excessive wing use (to aid in balance, locomotion, cooling, etc.) and activity (fear and escape behaviour). Consequently, husbandry practices should focus on limiting wing activity during grow-out, especially close to slaughter. Broilers tend to be flighty in response to high light intensities and increasing natural day length. Sudden and excessive wing use can be triggered by excessive human activity in the house (frequent penning and weighing birds, tilling litter, vaccinations, and flock thinning), unusual or novel sounds in and around the house/farm, as well as daily activities associated with access to feed and water, and attempts to perch or climb over migration barriers (i.e., especially plastic pipes used by some producers in the USA). In addition, broilers also extend their wings away from the body to facilitate convective heat loss. Proper ventilation during heat stress is of utmost importance in the prevention of DPM.

The influence of bird activity on other BMM is not well defined, although strains and micro-tears associated with muscular activity can overwhelm the repair processes. Bird handling and associated struggle during catching and crating can cause damage when excessive, but usually in the form of wing dislocations, muscle haemorrhages and blood splash.

Crating density, as well as heat stress during loading, transportation, and lairage are also very important in terms of metabolic exhaustion and muscle damage. It has been suggested that there is a role of feed withdrawal in the manifestation of BMM and carcass quality. In the event of prolonged feed withdrawal the muscle cells will exhaust glycogen reserves which means the muscle pH after slaughter is high which can result in DFD meat. Furthermore, prolonged feed withdrawal can cause a change in metabolism resulting in a catabolic state leading to yield losses. Recent internal Aviagen data showed that increasing feed withdrawal time by 4 hours increased the incidence of SS. It has also been noted that if feed withdrawal is too short the muscle can have excess glycogen stores which can result in excessive lactic acid build up in the muscle during transport and lairage resulting in a loss of integrity of the muscle and increased water loss; this is thought to increase the risk of SS or PSE. Therefore it is important that the recommended feed withdrawal time of between 8 – 12h before expected processing is maintained.

12.2. Slaughter

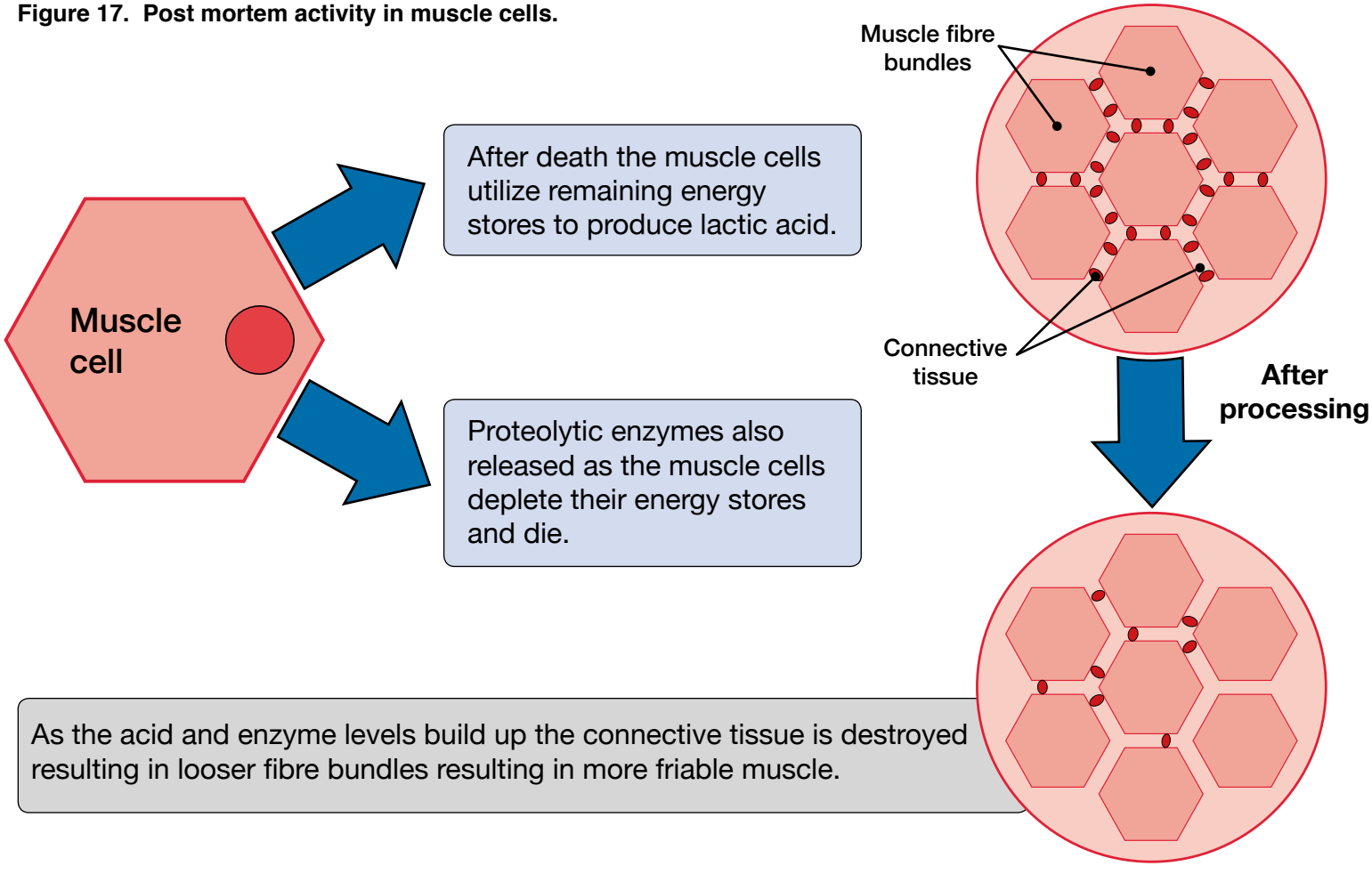
By definition, BMM are growth and muscle development associated structural lesions. Processes, such as stunning, bleeding, scalding, and de-feathering can only alter their physical manifestation. Little or no information is available on the effects of electrical currents (electrical stunning) or modified atmosphere (gas stunning) on BMM. The onset of rigor mortis (muscle rigidity) after slaughter can be accelerated by the stunning and electrical stimulation methods used. Voltage (>200 V), amperage, pulse frequency, duration, and location (pre- or post-defeathering) of electrical stimulation can affect extent of muscle (myofiber) damage and integrity from the severity of contractions (Sams, 2002). Also, difficulties in de-feathering frequently reported with the use of gas stunning systems (accelerated rigor of feather muscles) is typically over-compensated by higher scalding temperatures and plucking pressures, leading to skin and muscle tears.

Incorrect settings and/or poor maintenance of processing equipment has been observed to increase the severity and incidence of SS. For example, a comparison of two plants, one with and one without SS that were processing birds from the same production base found that the plant with high SS had a cooler scald temperature (47°C, 117°F) and a more aggressive plucker, as shown by a high incidence of broken wings.

12.3. Carcass chilling

The rate at which a carcass cools can have an impact upon meat quality due to the post mortem activity of muscle cells. After death the muscle cells produce lactic acid and release proteolytic enzymes which degrade connective tissue resulting in a softening of the meat (see **Figure 17**).

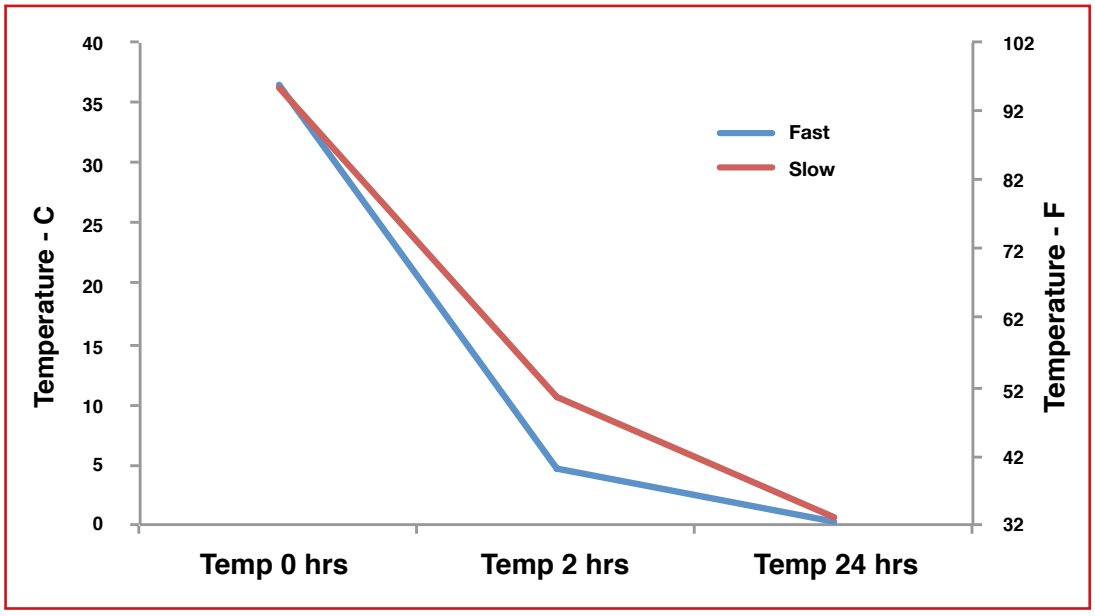
Figure 17. Post mortem activity in muscle cells.



This degradation by the lactic acid and proteolytic enzymes only occurs while the meat is warm. Consequently it is important that the carcass reaches target chill temp quickly to reduce connective tissue degradation.

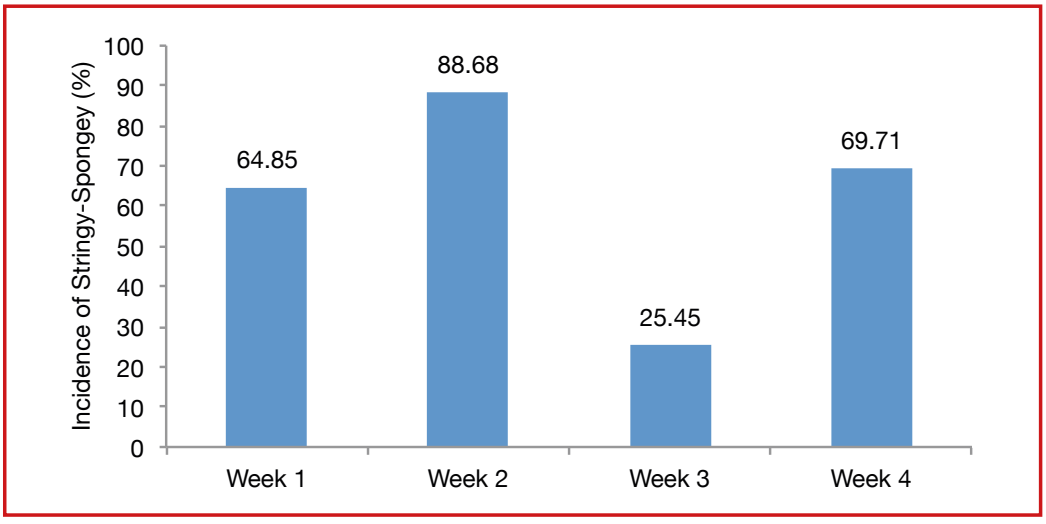
The impact of slower carcass cooling was examined in a trial by Aviagen where two rates of carcass cooling (air chilling) was examined within 4 consecutive groups of broiler chickens. **Figure 18** shows the mean temperatures obtained for the two cooling planes used for the birds in the trial (each week the birds were randomly split into two groups: one group were fast chilled and one group were slow chilled).

Figure 18. Difference in rate of chilling in a trial looking at the effect of chilling rate on incidence of BMM.



Following processing and cooling the carcases were assessed for incidence of BMM; the incidence of WS and WB were not affected by the cooling plane but SS was found to be higher in those birds on the slow cooling plane relative to their fast cooled counterparts **(Figure 19).**

Figure 19. Relative increase in incidence of stringy-spongy in slow-chilled birds.



This data shows the importance of adequate chilling after processing to prevent post mortem degradation of the muscle and potential loss of muscle integrity.

13. Appendices

13.1. Trial Designs

This appendix gives an overview of the Trial design for any Trial discussed in **Section 8** (Growth rate and BMM) not published in a peer reviewed Journal. Full description of the Trial methodology for other trials can be found in the appropriate published papers using the references given.

Trial 1. Quantitative Feed Control

	Quantitative Feed Control
Location	Aviagen Albertville, AL
Strain	Ross 708
Sex	Male
Trial period	0-48d
Lighting	23L:1d, 27 lux to 7d 20L:4d, 11 lux 8-48 d
Diets	Starter (1-12d, crumble) Grower (13-31d, pellet) Finisher (32-40d, pellet) Withdrawal (41-48d, pellet) Formulated to meet or exceed Aviagen recommendations Corn-soybean meal-based containing poultry by-product and distillers grains with solubles. All pens received ad libitum feeding for the first 24h after placement.
Treatments	**Control:** ad libitum **Trt 1:** 95% of control intake **Trt 2:** 90% of control intake **Trt 3:** 95% of control intake until 11d and then ad libitum (Early Control)
Records taken	BW and feed: 12, 31, 40 and 48d Processing: 32 and 49d Carcass components weighed and pectoralis major visually assessed and scored on a 3 point scale for WS and WB (0=none, 1=mild, 2=severe)

Trial 2. Qualitative Feed Control

	Qualitative Feed Control
Location	Aviagen Albertville, AL
Strain	Yield Plus x Ross 708
Sex	Male
Trial period	0-62d
Lighting	23L:1d, 25 lux to 7d 18L:6d, 10 lux 8-48d
Diets	Starter (1-11d, crumble) Grower (12-28d, pellet) Finisher 1 (29-40d, pellet) Finisher 2 (41-48d, pellet) Withdrawal (49-62d; pellet) Control was formulated to meet Aviagen recommendations for energy and AA Corn-soybean meal-based and contained in some cases containing poultry by-product and distillers grains with solubles. Wheat middlings were incorporated to achieve desired nutrient dilution All pens received ad libitum feeding.
Treatments	See table below
Records taken	BW and feed Processing Carcass components weighed and pectoralis major visually assessed and scored on a 4 point scale for WS and WB at day 63 (0=none, 1=mild, 2=moderate, 3=severe)

Treatment	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Last day fed	11	28	40	48	62
1	Control	Control	Control	Control	Control
2	Low	Control	Control	Control	Control
3	Low	Low	Control	Control	Control
4	Low	Low	Low	Control	Control
5	Low	Low	Low	Low	Control
6	Control	Low	Control	Control	Control
7	Control	Low	Low	Control	Control
8	Control	Low	Low	Low	Control
9	Control	Control	Low	Control	Control
10	Control	Control	Low	Low	Control
11	Control	Control	Control	Low	Control
12	Low	Low	Low	Low	Low

Control = 100% of Aviagen nutrition specifications for amino acids and dietary energy base.
LOW - 90% of Aviagen nutrition specifications for amino acids and dietary energy base.

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