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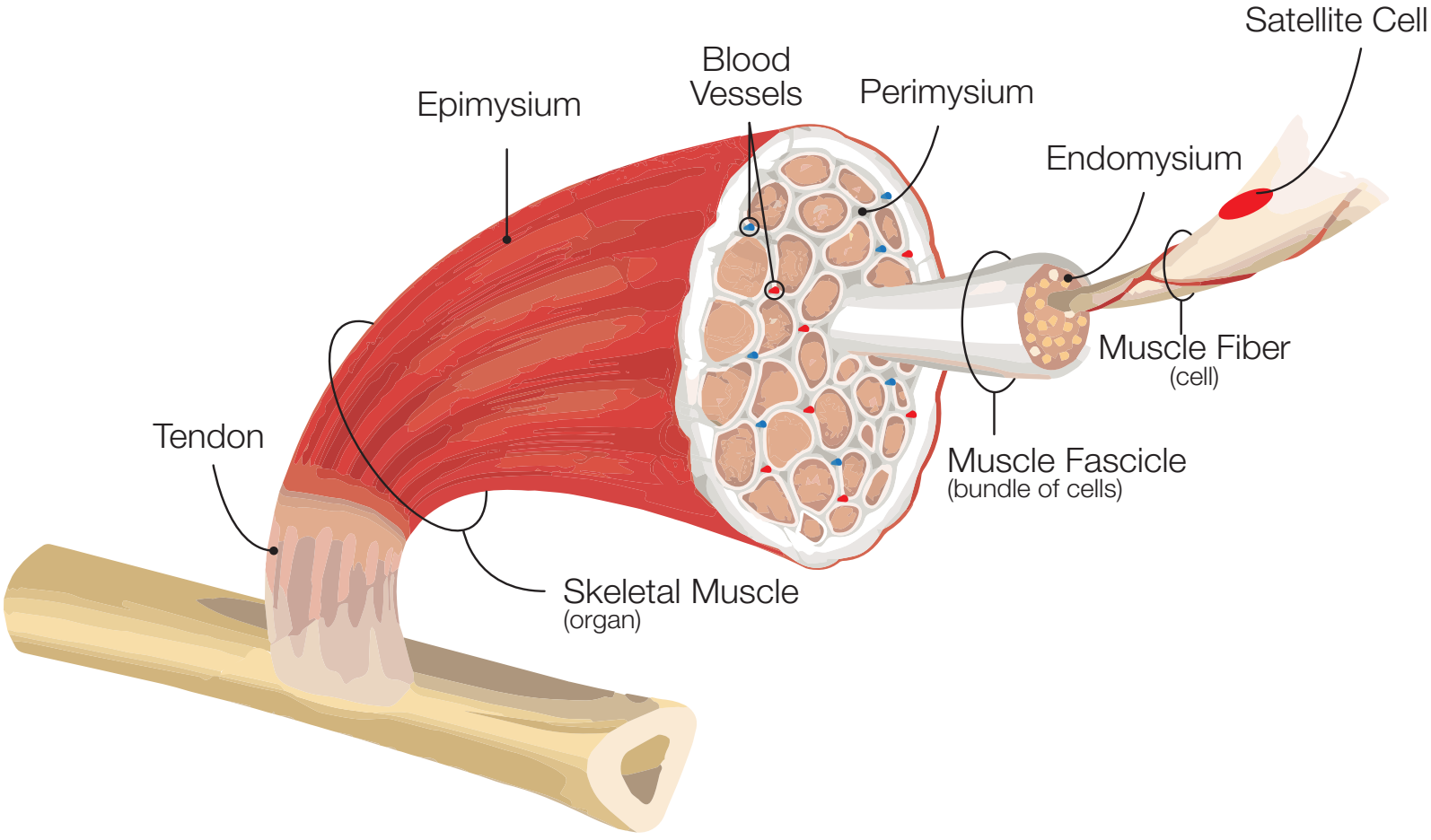


Broiler Myopathies Handbook

Contents

1. Overview

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

1.1. Actions that may reduce incidence and severity of broiler myopathies (BM)

- Focus on good brooding management, good minimum ventilation (do not allow CO_2 levels > 3000 ppm), good feed quality and good access to feed.
- Obtain good growth in the first 10 d as this period is important for muscle development. Target growth to achieve a body weight of at least 4 times placement weight by 7 d.
- Prevent sudden growth acceleration (>120 g or 0.26 lbs/d), for example, after flock thinning.
- Minimize flock disturbances and prevent excessive wing flapping.
- Prevent high body temperatures in broilers 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. Pay close attention to amino acid levels higher than Aviagen advice.
- There is strong evidence that modifying the growth profile can reduce the incidence of BM, although there may be some loss in broiler performance. Modifying the growth curve can be achieved by:
  - Reducing the quantity of feed provided to the birds to 97% of the feed of ad libitum fed birds. It is recommended that this is done between 15-32 d.
  - Reducing lysine levels in the diet to 85% of Aviagen advice during the period when birds are growing the fastest (15-32 d) has been found to significantly reduce BM with minimal 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 processing. Treatment should not start before 14 d 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 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 (SS) muscle, then look at scalding temperatures, de-feathering and carcass chilling practices in the processing plant.

2. Introduction

Over the last several years there has been an increase in the number of reports of BM 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 BM 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 resources into researching the subject, as have several university researchers.

The first version of the BM Handbook was released in 2019 and provided a summary of Aviagen's investigative work into understanding the physiology, metabolic causes and factors involved that influence the incidence and severity of BM. Since the release of the first handbook, understanding of myopathies and the multifactorial influences involved has grown, both in the depth of knowledge surrounding breast myopathies but also other myopathies not specific to the breast muscle. Likewise, the volume of data collected from trial and field data has allowed Aviagen to focus further investigative study to complement the existing research and explore new areas.

The purpose of this revised version is to update and complement the previous publication by incorporating findings of latest investigative research into the subject of broiler myopathies. Many of the points highlighted in the first BM Handbook remain as core content; further detail on subjects such as processing and nutrition are also included. The content now extends to myopathies that are not specific to the breast muscle such as Doral Cranial Myopathy.

Not all of the myopathies discussed have a major effect on product quality, but are included for completeness.

2.1. History of BM

The first important broiler 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 1960s. DPM manifests as one (or both) of the inner breast fillets (i.e., pectoralis minor) atrophying and turning green. The condition was seen in broilers in the late 1990s and is still occasionally observed in processing plants.

In the 1990s, 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-processing stress from the time of catching the birds to go to the processing plant and the actual processing. 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 BM 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 BM

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. It should be noted that it is unlikely that the incidence of BM will reach zero solely due to genetic selection, as non-genetic factors also affect the incidence of myopathies **(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 BM. Aviagen has undertaken a wide range of trials on factors that could affect the incidence of BM, looking at nutrition, management, incubation practice and genetics. Aviagen has also collaborated with universities and companies undertaking research on BM. The results from these trials and collaborations will be reported within this handbook.

3. History of BM

3.1. Broiler types affected by BM

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

3.2. Distribution of BM 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 BM 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 BM upon veterinary health inspections during processing varies. In general, BM are considered a quality issue, not a food safety issue (Bilgili, 2016). To date there has been no evidence of bacterial or viral contamination **(Section 10)**. Most of the product with BM is sorted and either condemned (DPM) or diverted to alternative uses. However, if the BM is severe and accompanied by inflammatory signs (i.e., focal pin-point hemorrhages, gelatinous fluid, etc.), then 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 BM. The amount of muscle trimmed and/or discarded because of BM 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 BM, and especially of DPM, in whole carcass markets. As for the mild forms of BM, the sorted product is often diverted to alternative market segments (e.g., further processing) that allows for some value recovery.

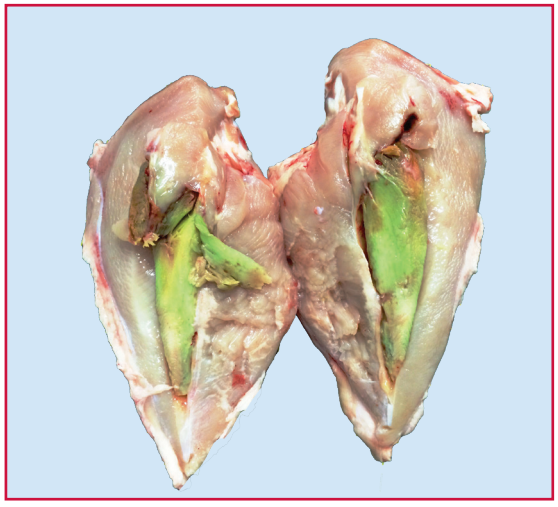
4. Histology of BM

4.1 Internal pectoral myopathy

4.1a Deep pectoral myopathy (DPM)

This condition, first reported in broiler chickens in the 1980s, 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 hemorrhagic 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 the 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 (ischemic necrosis) of the muscle fibers. 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.

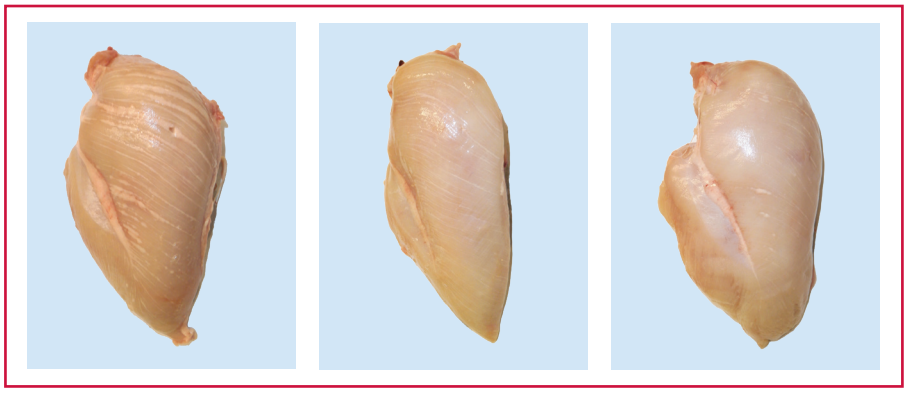
4.1b Tender feathering (TF)

Tender feathering is characterized by a loss of integrity of the Pectoralis minor muscle. Histological analysis has shown that there are no significant differences in the structure of the muscle in tenders with and without feathering. One hypothesis for TF is that a lack of connective tissue in the muscle increases the risk of it becoming friable. This theory was investigated by quantifying the hydroxyproline content of inner pectoral muscles with and without feathering; hydroxyproline is a component of collagen, and gives an indication of the integrity of the connective tissue in the muscle. The results showed no significant differences in hydroxyproline between the affected and unaffected muscle samples, indicating that TF is not linked to reduced connective tissue in the Pectoralis minor muscle. Examination of the different methods for removing the tenders from the carcass have shown that TF is more likely with certain techniques. The use of antimicrobial compounds such as peracetic acid (PAA) may also pose a risk to the development of TF. While PAA helps to minimize the risk of bacterial contamination from chicken meat, prolonged exposure to PAA can have an impact on the connective tissue in the meat causing disruption to the collagen fibers. The method in which the tenders are stripped from the carcass or exposure to antimicrobial compounds such as PAA may increase the risk of tender feathering. The occurrence of TF seems to be related to challenges to the physical integrity of the muscle, and not to developmental reasons.

4.2. White striping (WS)

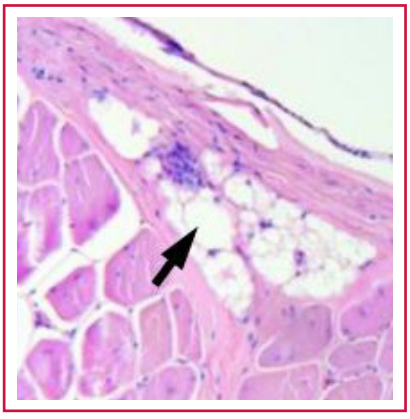
Reports of WS in broiler chickens have increased in recent years. This condition primarily affects the Pectoralis major muscle, and is characterized by visible white lines parallel to the direction of the muscle fibers; the quantity and thickness of the white stripes can vary from bird to bird (**Figure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

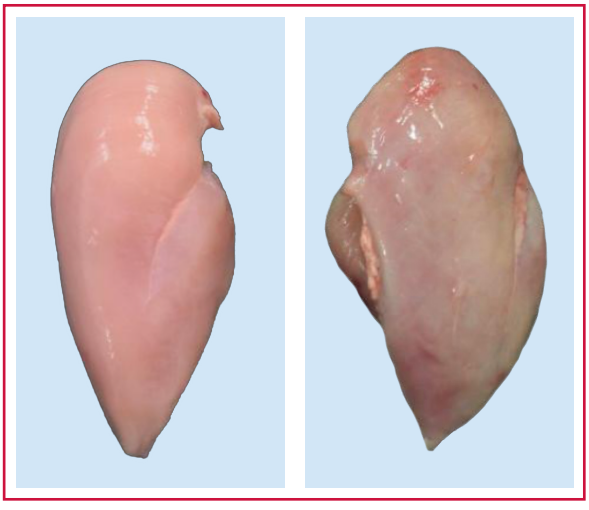


It has been reported that breast tissue severely affected by WS can exhibit an increase in connective tissue with varying degrees of muscle fiber degeneration and regeneration at the microscopic level. All muscle tissue will normally show some level of muscle fiber degeneration and regeneration, but 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 fiber. The exact cause of WS is still not known and understanding this condition is an active area of research.

4.3. Wooden breast (WB)

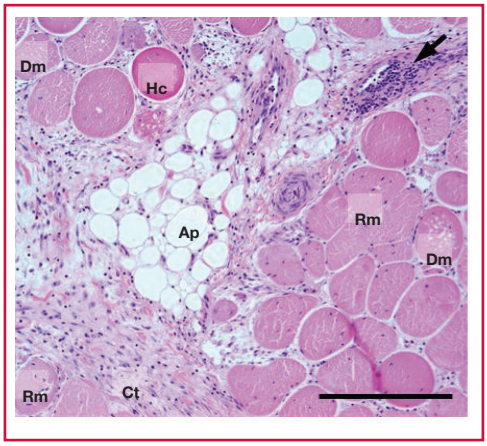
This myopathy also affects the Pectoralis major muscle and is characterized by a hardening of the breast muscle typically in the thicker part of the fillet. However, the hardening can be found throughout the muscle in more severe cases. Depending on the severity of the condition, other visual features of WB include a paler color, surface hemorrhaging 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 fibers, hypercontracted fibers, 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 fibers (Dm), regenerating fibers (Rm), adipose tissue (Ap), hypercontracted fibers (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 development.

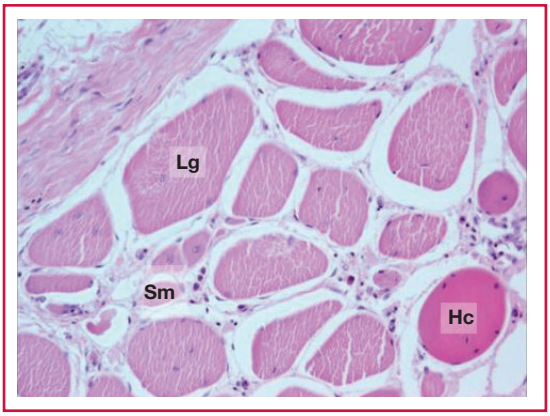
4.4. Stringy-spongy (SS)

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 fibers. 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 fiber 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.

Figure 6. Image of fillet with stringy-spongy.

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

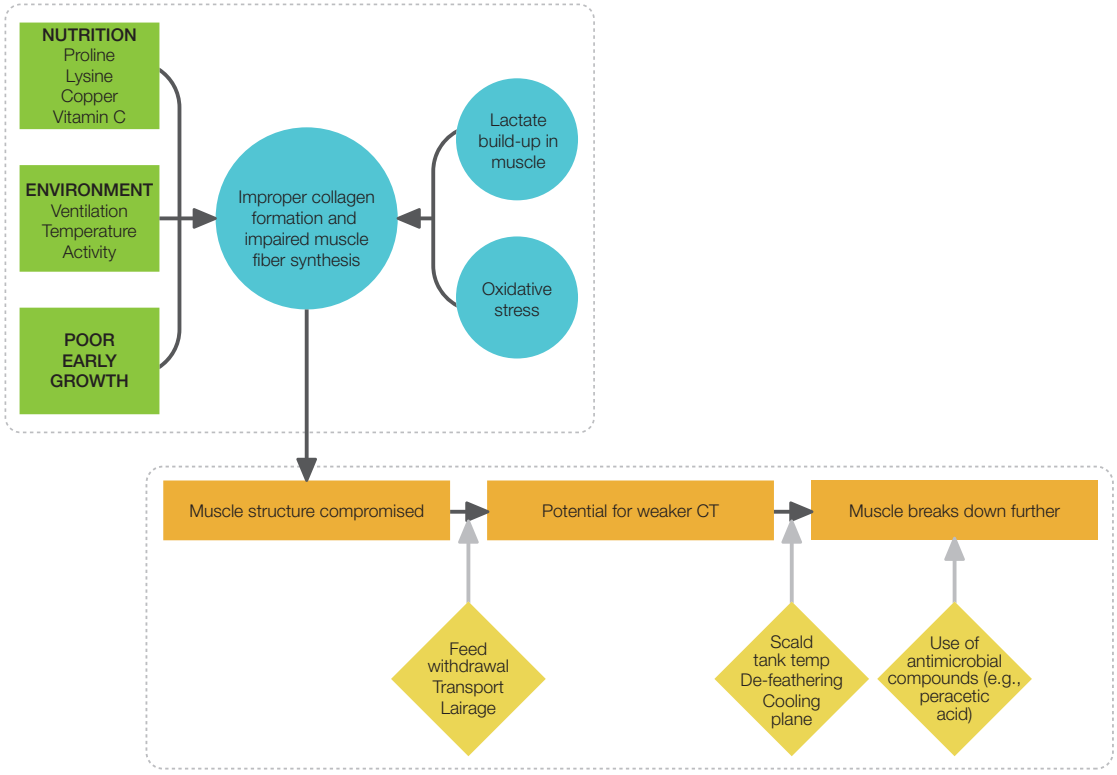
Figure 7. Histomicrograph of breast muscle affected by stringy-spongy. Features of the muscle include large (Lg) and small (Sm) muscle fibers along with hypercontracted fibers (Hc), (100µm).



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 fibers and bundles together. Additionally, increased levels of lactic acid can also inhibit protein synthesis which may also impact the maturation and thus integrity of the connective tissue in the muscle.

When the pH drops in the muscle it can cause lysis of the muscle fibers which results in the release of proteolytic enzymes into the extra cellular space. These enzymes can 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 fibers (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. **Figure 8** provides insight into the components that may play a role in the development of SS.

Figure 8. Factors contributing to the development of SS.



To date, Aviagen has been unable to identify SS on farm immediately post-mortem. However, if birds are euthanized and left to cool at ambient temperature SS can sometimes be seen to be developing a couple of hours after. This supports the hypothesis that it is linked to post mortem changes in the muscle and the rate of carcass cooling.

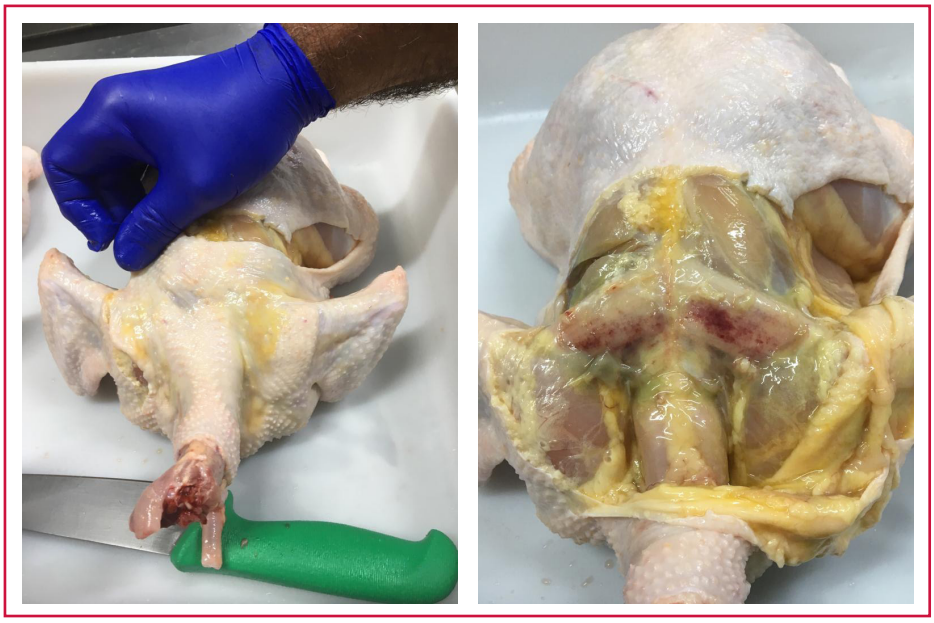
As part of the investigations into the biological basis for SS Aviagen quantified the level of hydroxyproline present in muscle samples with and without SS. Hydroxyproline is a key component of collagen which gives the strength and integrity to the connective tissue within the muscle. Our results showed that as SS severity increases a reduction in hydroxyproline content is seen. This indicates that P. major muscles with a reduced hydroxyproline content are more predisposed to developing SS during processing.

De-feathering time and intensity have been identified as risk factors for the manifestation of SS. If the carcasses are de-feathered too intensely or for too long there can be a loss of muscle fiber integrity. Scald temperature should also be considered as a risk factor for SS. When the plucking scald temperatures are low more intense de-feathering is required to remove the feathers which will impact upon the integrity of the muscle.

4.5. Dorsal Cranial Myopathy (DCM)

Dorsal cranial myopathy (DCM) is a condition which affects the anterior latissimus dorsi (ALD) muscle of chickens. The ALD is located between the base of the neck and the wing joint. This muscle is similar to the mammalian trapezius muscle, and its major function is to draw the wings upwards into a tucked position so that they don't droop towards the ground when the wings are not in use. During contraction of the pectoralis major and minor (supracoracoideus) muscles, the ALD is responsible for governing the movement of the humerus. Dorsal Cranial Myopathy typically appears as pale ALD muscles with some hemorrhagic areas and may be thickened with evidence of fibrosis and necrosis. The lesions are usually bilateral and the areas around the affected muscle can be edematous and a yellow citrine-colored fluid may be present (**Figure 9**).

Figure 9. Lesions from DCM affected birds.



Photos courtesy of Dr. Sarge Bilgili, Professor Emeritus, Auburn University Department of Poultry Science

Reports of DCM are not new; it was first reported in 2008, but when observed, is sporadic in incidence and generally without specific data or detail to allow an assessment of causal or contributing factors. Observations of DCM have been reported in many regions globally, and the incidence is generally low (<1.5%). There is some evidence that the risk of DCM increases with increased body weight and that males have a greater incidence than females (Zimermann et al., 2012).

This myopathy is usually only reported at the processing plant, as it is not easy to detect through the skin of the live bird, and can be missed during routine post mortem inspection. It is typically seen in heavier birds, however it has been speculated that an increased processing age gives more time for the lesion to develop and become visible. In the field there are no clinical signs that have been associated with DCM, which can make detection difficult at flock level. The exact etiology of DCM is unknown; however, the histological features suggest hypoxia or oxidative damage in the muscle. There have been a number of hypotheses, with some suggesting it could be an exertional myopathy similar to DPM, and others suggesting a nutritional myopathy linked to an insufficiency in dietary antioxidants such as vitamin E and/or selenium. Toxicity from ionophores and copper has also been implicated as a potential cause for DCM. Even though there are some similarities in histological presentation, there is no evidence that DCM is linked to other myopathies such as WB or WS.

While more research is needed to fully understand the etiology of this condition, this note is aimed to provide an overview of the knowledge available about DCM and highlight potential risk factors that could contribute to the field incidence and severity.

Similar to other broiler myopathies, DCM is a product quality issue and does not represent any health risks to the consumer.

5. Impact of BM on Eating Quality

Table 1 summarizes the effects of different BM on product quality and usability in the processing plant. As noted earlier, BM 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 BM 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 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 analyzer (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 researchers 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 liked 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 minimal research on consumer preference or eating quality of DFD breast meat.

5.6. Deep pectoral muscle myopathy (DPM)

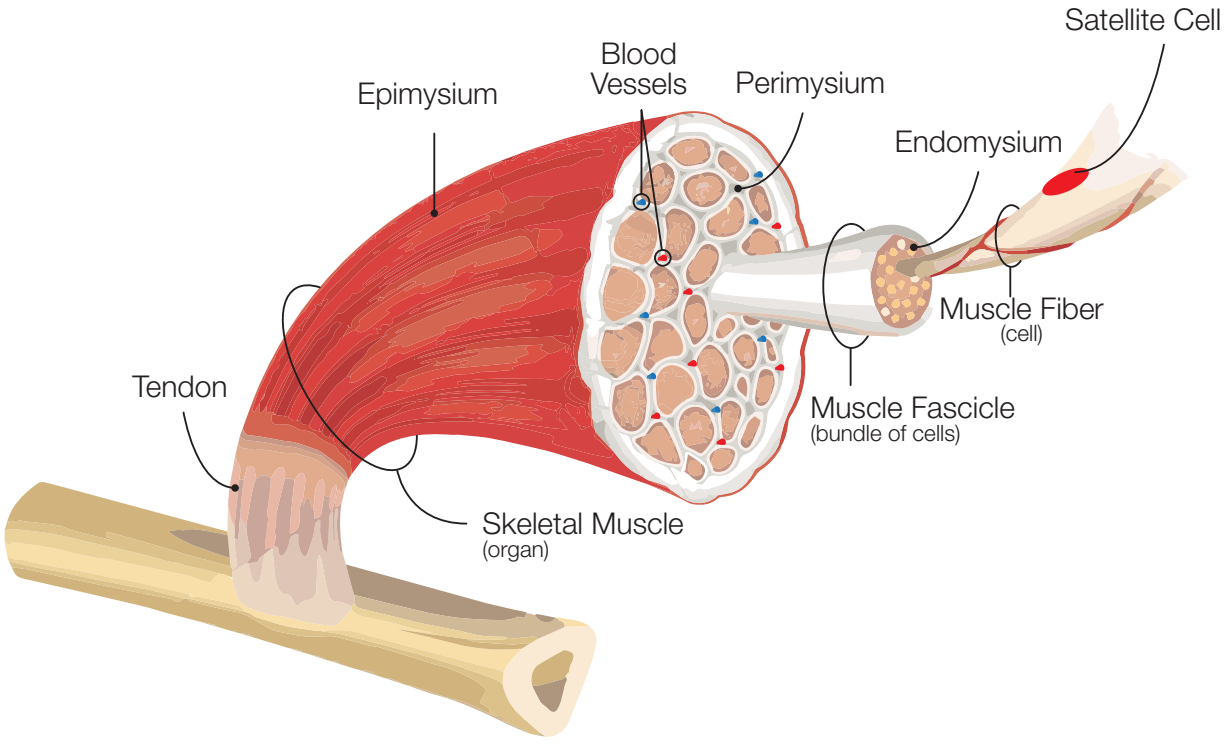
The muscle fillet affected by DPM is rejected for human consumption and the remainder of the carcass may be trimmed to recover the unaffected meat. The largest issue is for the whole bird market where the problem is found by the consumer.

6. Basic Muscle Biology

6.1. Structure and function

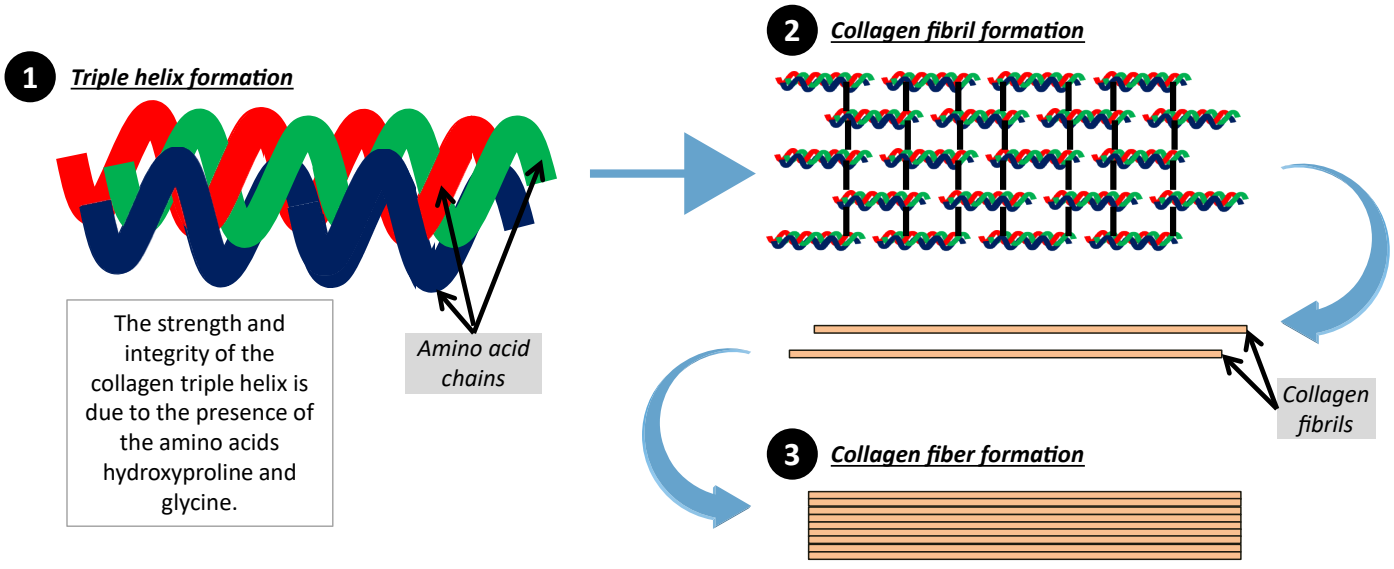
Muscle tissue is comprised of 75% water, 20% protein, and the remaining 5% of fats, carbohydrates and minerals. Skeletal muscles attach through bundles of collagen (tendons) to the bones of the skeleton to facilitate body structure, posture and movement, and thermoregulation. The skeletal muscle is composed of bundles (fascicles) of muscle fibers covered by layers of connective tissue sheet (endomysium, perimysium and epimysium) which is composed of collagen and other glyco-proteins (**Figure 10**). 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 10. Compenents of the skeletal muscle.



The connective tissue matrix surrounding the muscle is an intrinsic part of the overall integrity of the muscle. The main structural component of the connective tissue layers is collagen and it strength comes from the structure and interaction of the collagen molecules. Collagen is made up of predominately of the amino acids proline, glycine, hydroxyproline and hydroxylysine bound together tightly in a triple helix (**Figure 11**). These helices are then linked together to form collagen fibrils which are then further bound to form the collagen fiber.

Figure 11. Collagen structure. Collagen is a complex molecule and its core structure is a triple helix comprising of three single helical chains of amino acids tightly woven together (1). The triple helices join together in long chains to form collagen fibrils (2) which further join together to form the strong collagen fibers (3) which then form connective tissue. Formation of strong triple helices and robust collagen fibrils depends upon the correct supply of amino acids in the diet along with adequate vitamin C, copper and iron. The biological processes involved in collagen fiber formation require oxygen therefore optimal ventilation in the chicken shed is essential.



The collagen fibers can be bound together in long rope like structures which run parallel to the muscle fibers and hold them together by forming anchors to the fibers at regular intervals. Alternatively, the collagen fibers can form mesh like sheets which wrap around the muscle fibers and muscle fiber bundles to keep them tightly bound and secure. The integrity of muscle therefore relies on optimal collagen formation. Research shows that vitamin C, copper, iron and oxygen, along with the correct amino acid provision, are essential nutrients for the formation of strong structural collagen.

Muscle fibers are 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 fibers 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 dependent) 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 fiber 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 categorized 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 fiber types. The pectoral muscles (major and minor) of chickens are composed of white muscle fibers, 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 fibers 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 fibers. Embryonic muscle increases in size through proliferation of newly formed muscle cells which increases the number of muscle fibers (hyperplasia). After hatch, the muscle only increases in size though the enlargement of the muscle cells (hypertrophy). Muscle fiber 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 fibers 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-signaling molecules, hormones and growth factors. The satellite cells proliferate and the new cells fuse together to repair the damage and form new myofibers. The ability of a satellite cell to divide and proliferate is finite, which means their activity decreases 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 fibers compared to white muscle fibers, as red muscle undergoes more maintenance and repair from daily activities.

6.4. Muscle changes post-processing

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 processed. The amount of lactic acid produced post-mortem depends upon the level of glycogen in the muscle (influenced by pre-processing 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 BM

The exact cause(s) of BM 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).

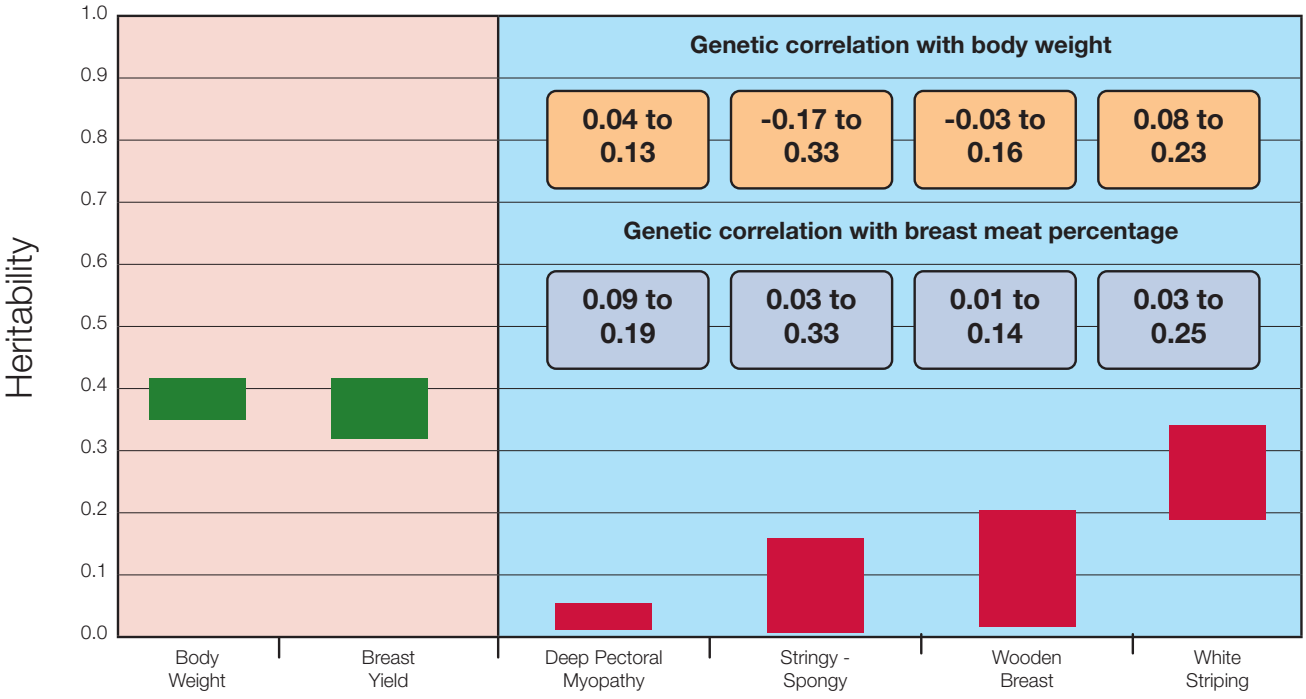
This may be especially critical during periods of high metabolic activity (e.g., exertion or over-stretching of breast muscle). The build-up of lactic acid and lack of sufficient oxygen can result in loss of muscle cell membrane integrity, depletion of energy and increase in intracellular calcium leading to skeletal muscle cell hyper-contractility and, ultimately, cell death. When there is damage to a muscle fiber 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 less stable 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 BM. 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 BM

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; Bailey et al., 2020). The range of heritabilities of breast muscle myopathies are displayed, along with those for breast yield and body weight for comparison, in **Figure 12**.

Figure 12. Heritabilities of BM, body weight and breast yield (Bailey et al., 2015; Bailey et al., 2020).



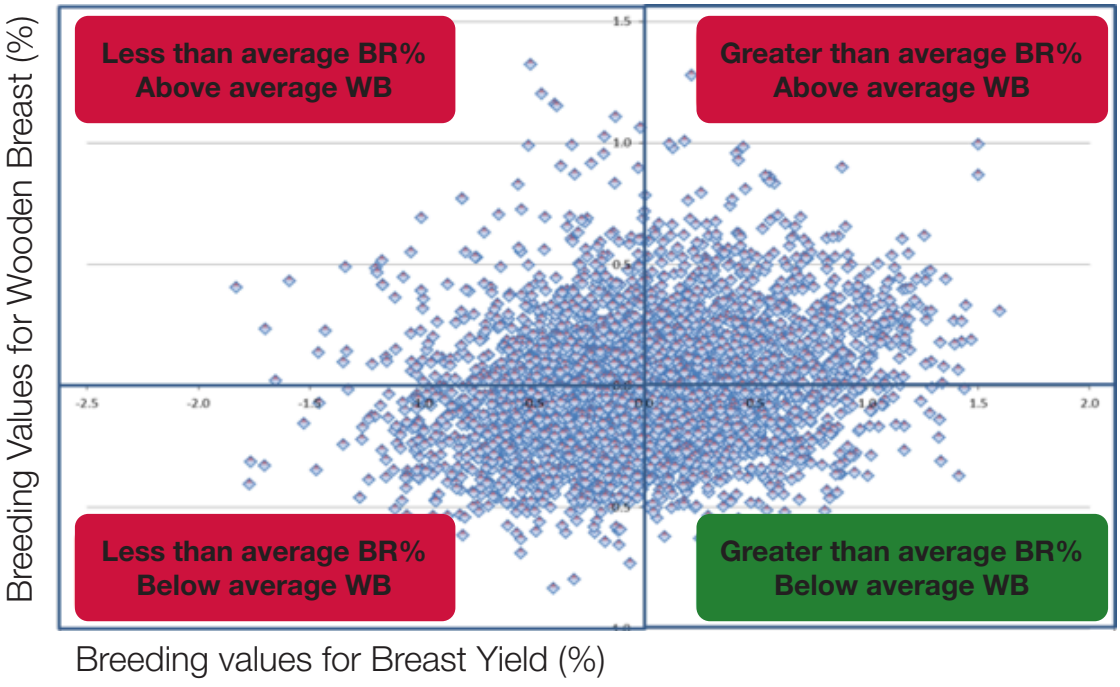
Every trait in the chicken is under the control of genetic factors (passed down from the parents) and environmental factors (management, nutrition, gut and immune challenge, house environmental conditions, climate, etc.) and the sum of these effects results in the observed field performance. Heritability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

Figure 12 compares the range of heritability estimates of body weight and breast yield (green bars) with those of BM (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 BM, indicating the influence of non-genetic factors is much greater for BM. The heritabilities for DPM, SS and WB 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 BM; 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; for example, does genetic selection for one trait result in a correlated genetic response in another trait. The chart in **Figure 12** shows the genetic correlations between body weight and breast yield with BM. Body weight was estimated to have a genetic correlation between -0.170-0.228 with BM, and breast yield a genetic correlation of between 0.092-0.330 with the BM. The low genetic correlations described indicate there is a low level link between increased breast meat yield and body weight, and expression of BM. While it has been suggested that the incidence of BM 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 BM. The scatter plot, below (**Figure 13**), 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 BM as part of a balanced breeding program, aiming to reduce the genetic propensity to express BM in the field. Given the relatively low genetic basis of BM, genetic selection will yield small to moderate improvements in the expression of BM. It is clear that the key to reducing the expression of BM 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 BM.

Figure 13. 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, fibrosis, immune cell infiltration, hypoxia, fat deposition and muscle fiber 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 BM. The information on the genetic basis and genetic correlations of the BM with other traits can then be included as part of a balanced breeding goal as described above.

7.2. Metabolomic and proteomic analysis

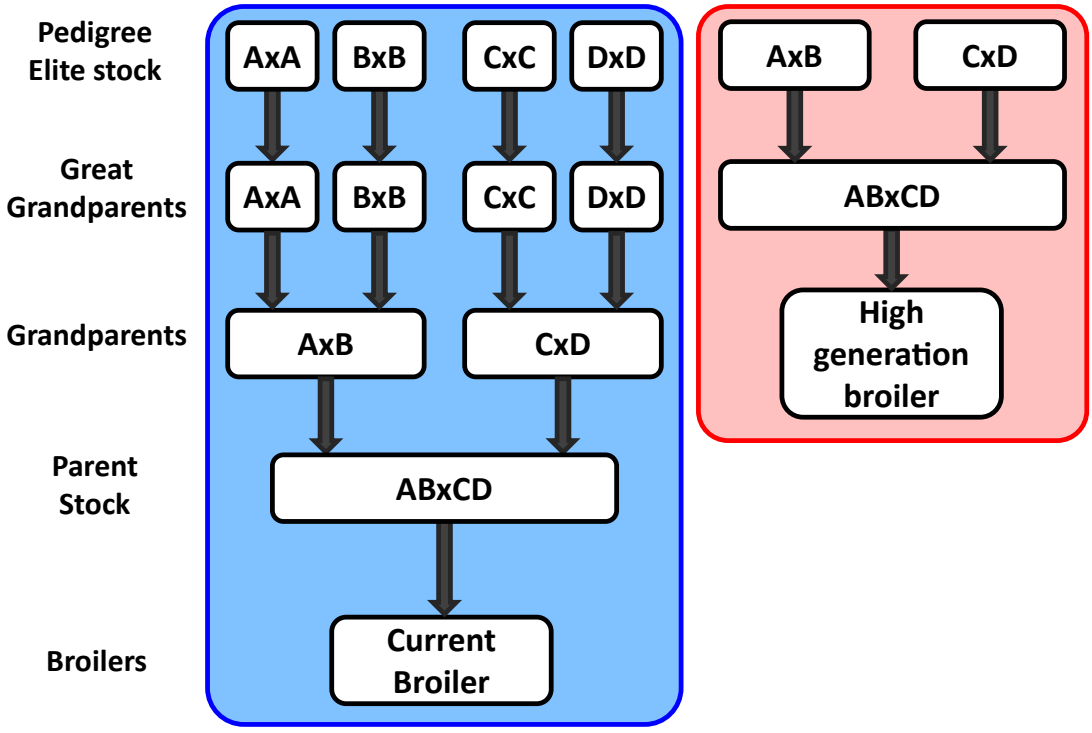
Further investigations to understand the underlying mechanisms of BM include the use of metabolomics and proteomics to characterize the cellular and physiological differences between breast fillets with and without myopathies. Kuttappan et al. (2017) demonstrated, through the use of proteomics, that 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) analyzed 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.

7.3. High generation trials

As part of Aviagen's ongoing efforts to reduce the genetic propensity for WB there is a continuous assessment of selection candidates and evaluation of carcasses of siblings for the presence and severity of WB. This information enables the prediction of breeding values for the genetic propensity to develop WB; those birds with a reduced genetic propensity to develop WB are then eligible for succession in the breeding program.

In order to empirically demonstrate the impact of genetic selection on the incidence of WB, Aviagen generated a "high generation" (HG) broiler by fast-tracking the generation of PS from the mating of birds from the GGP generation, as described in **Figure 14**.

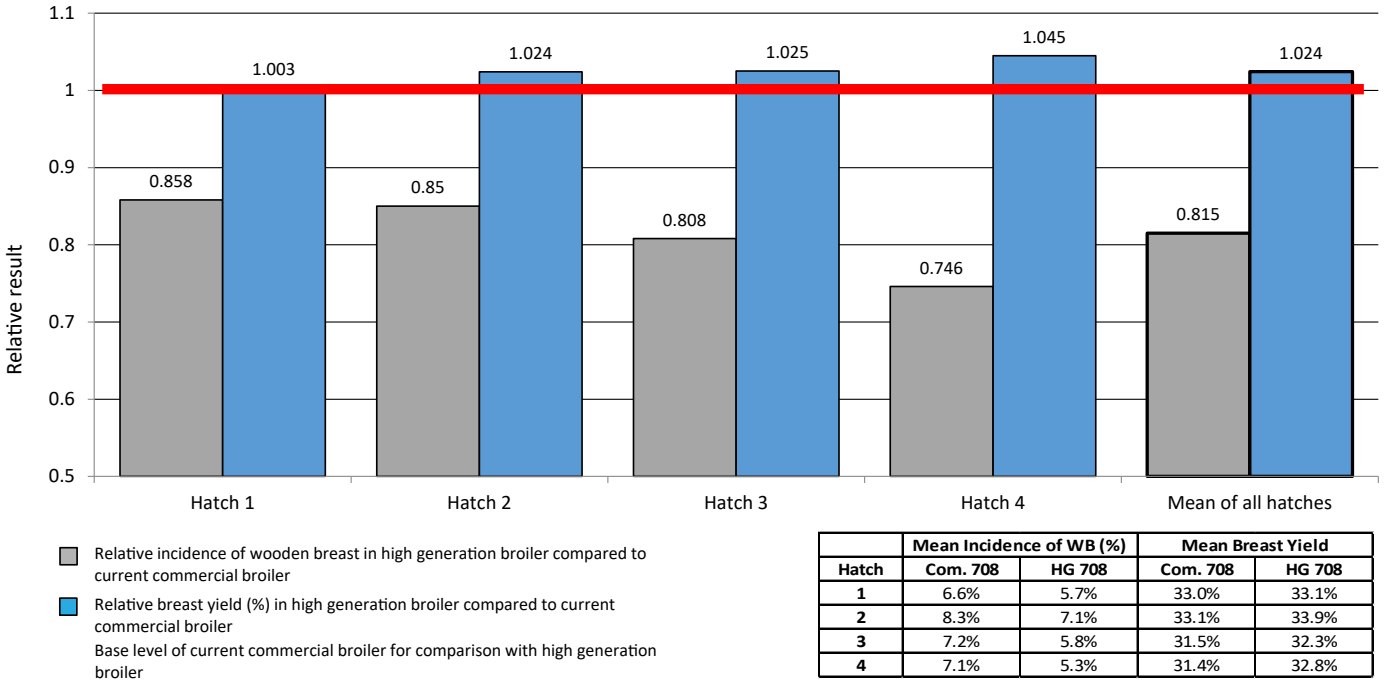
Figure 14. Development of high generation (HG) broilers.



The impact of genetic selection against the genetic propensity to WB was then assessed by comparing the performance of the HG and the current commercial broiler. In this study, hatching eggs for commercial Ross® 708 broilers were incubated alongside hatching eggs for HG Ross 708 broilers as per Aviagen recommendations. The chicks of both groups were reared side by side in separate pens as commercial broilers as per the ***Broiler Management Handbook.*** A total of four consecutive hatches were placed, and a sample of the birds of each group, commercial and HG broiler, were sent for processing. Hatching eggs for the HG broilers originated from the same parent flock for each of the four consecutive hatches, whereas hatching eggs for commercial broilers were obtained from commercial field flocks, age-matched where possible. PS for both commercial and HG broilers were fed feed from the same feed mill, and the same management parameters were used during the rear and lay as per the ***Parent Stock Management Handbook.*** Birds from both populations were processed; breast yield was measured and the birds were evaluated for the presence of WB.

In each hatch, a reduction in the relative incidence of WB can be seen in the HG broiler compared to the current commercial broiler (**Figure 15**). Simultaneously, there was a relative increase in Breast Yield (BY) seen in the HG broiler in each hatch compared to the current commercial broiler. Overall the higher generation broilers had on average 18.4% less WB ($p < 0.001$) and 2.4% more breast yield ($p < 0.03$) relative to the current commercial broiler. These results demonstrate that selecting increased breast meat in broilers can be achieved while selecting against the genetic propensity to develop BM such as WB. The generational difference between the commercial and HG broilers used in this study represents two years. An annual realized relative reduction in WB of 9.2% and an annual realized relative increase in BY of 1.2% displaces the theory that increased BY directly influences and increases the incidence of WB.

Figure 15. Incidence of WB in high generation broilers vs. current commercial broilers.



	Mean Incidence of WB (%)		Mean Breast Yield	
Hatch	Com. 708	HG 708	Com. 708	HG 708
1	6.6%	5.7%	33.0%	33.1%
2	8.3%	7.1%	33.1%	33.9%
3	7.2%	5.8%	31.5%	32.3%
4	7.1%	5.3%	31.4%	32.8%

8. Growth Curve and BM

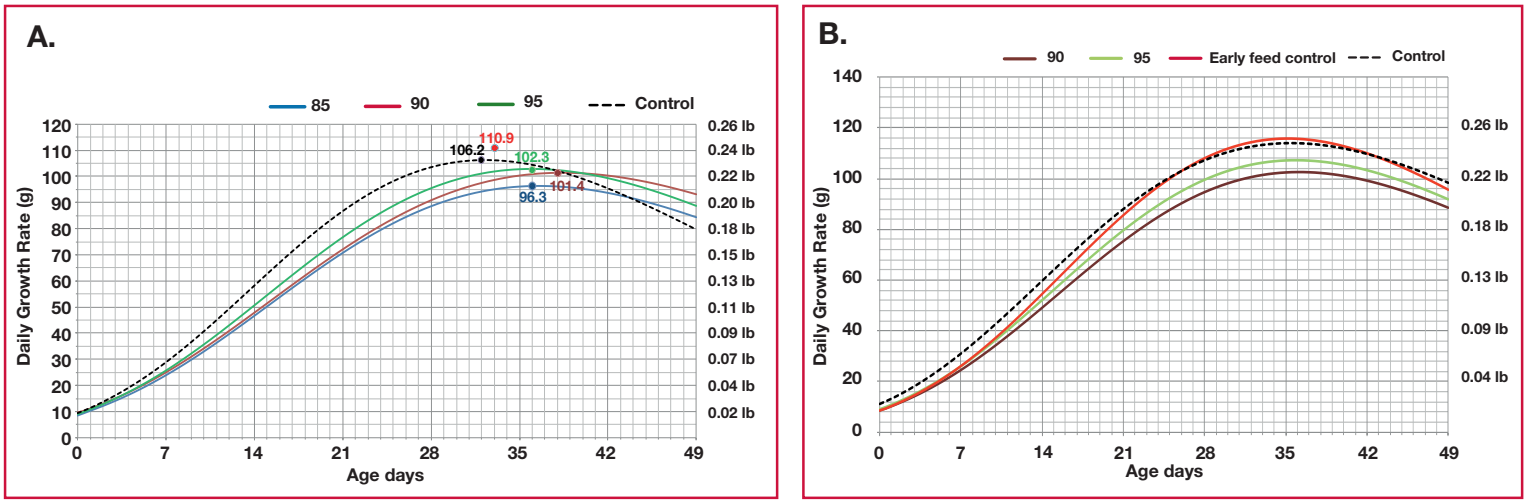
As part of early investigative work, field observations from Western Europe suggested that sudden growth accelerations such as those occurring post-thinning (which results in abrupt increases in available feeder, drinker and floor space for the remaining birds) increased the incidence and severity of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

With little scientific evidence available to support or refute this claim, Aviagen and Auburn University began a collaborative Ph.D. 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 BM.

8.1. Modifying growth using quantitative feed control

The effect of controlling feed intake and thereby modifying the growth curve on the incidence on BM 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 16**). Meloche et al. (2108a) concluded that controlling feed by 95% of ad libitum throughout the life significantly reduced BM 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 16. 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 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 d and processed at 46 d. Similarly, feeding 95% of ad libitum from 0-11 d 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 (0-11 d) is not as effective in controlling BM 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. Where farms are equipped with equipment that can measure and control feed intake, it can be a method of reducing the incidence of BM, although this should 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 BM 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 BM. 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 BM (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 BM 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 8-14 d and 8-25 d (Meloche et al., 2018b, Experiment 1) and 0-11 d and 0-28 d (internal Aviagen trial) increased the incidence of WB and WS, supporting the observation that poor early nutrition may increase the risk of BM (**Section 10.2**). It was concluded that reducing diet nutrient density was not an effective method of reducing BM.

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

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}	

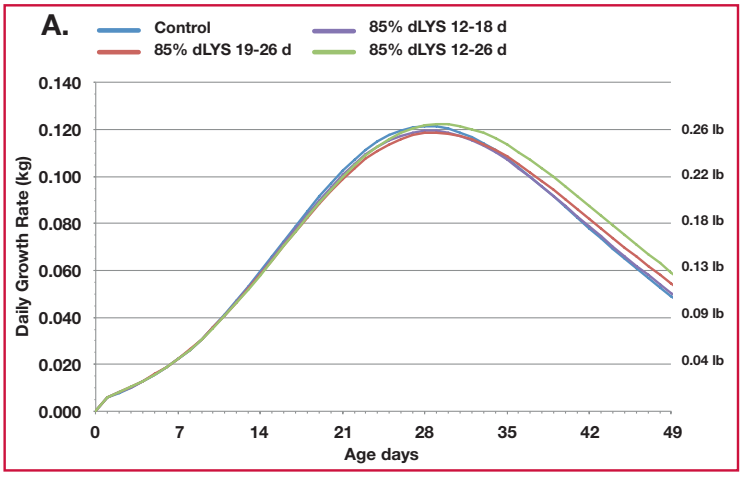
1. Birds were fed diets either as Aviagen recommendation (100%) or all nutrients were reduced to either 95 or 90% of Aviagen recommendations.
2. Results within a box with the same superscript are not significantly different at p<0.05. Where no superscript is shown there were no significant differences.
3. Feeding periods were 8-14, 15-25, 26-42 and 43-48 d.
4. Feeding periods were 0-11, 12-28, 29-40, 41-48 and 49-62 d.
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.

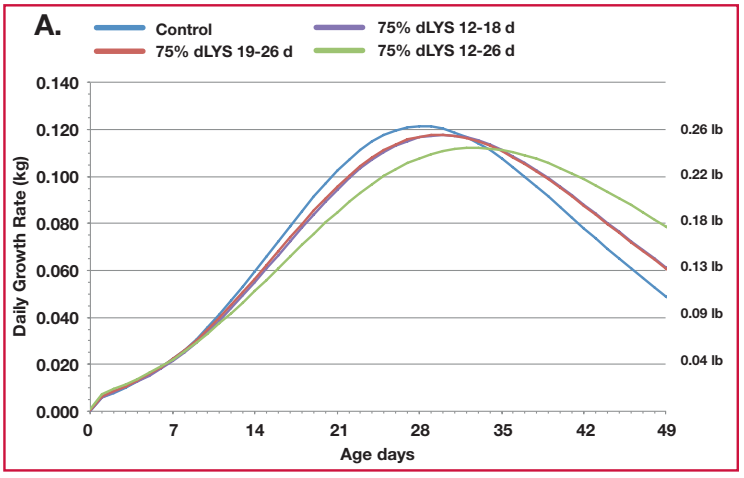
8.3. Lysine deletion

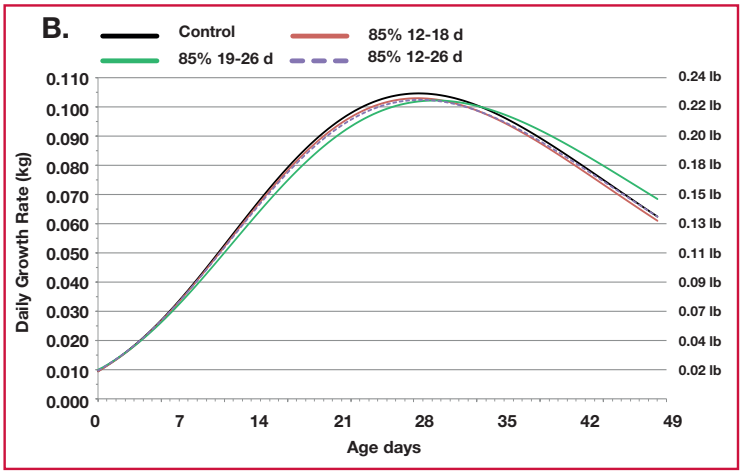
In order to be most effective, a qualitative approach to feed control must not result in the broiler increasing voluntary feed intake to compensate for the change in dietary nutrient density. One possible approach is to reduce the dietary concentration of a single critical amino acid (AA). If an AA is 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 AA content of feather-free carcass protein but only ~ 2% of the AA 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 mill.

Meloche et al. (2018c, d) showed that reducing only the dietary dLYS levels below Aviagen recommendations could alter the growth curve (**Figure 17**) and significantly reduce the incidence and severity of both WB and WS (**Table 5**). At early processing ages (41-48 d), a 75% reduction in dLYS in diets fed between 12-26 d reduced the incidence of severe WB and WS, although there was also a significant reduction in breast meat yield (Meloche et al., 2018c, d),reduced body weight and increased FCR (Meloche et al., 2018d). Broilers processed at a later age (61 d), 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 17. 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 15-32 d to male and female broilers that were processed at 58 d (**Table 5**). The incidence of WB and WS was very low in the females and 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% 12-18 d 85% 19-26 d 85% 12-26 d 75% 12-18 d 75% 19-26 d 75% 12-26 d	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% 12-18 d 85% 19-26 d 85% 12-26 d	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% 15-25 d	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% 15-25 d	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% 15-32 d	58	0.4 0.4	0.9 0.0	3.508 (7.73) 3.576 (7.88)		29.25 29.59
	Males	Control 85% 15-32 d	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.

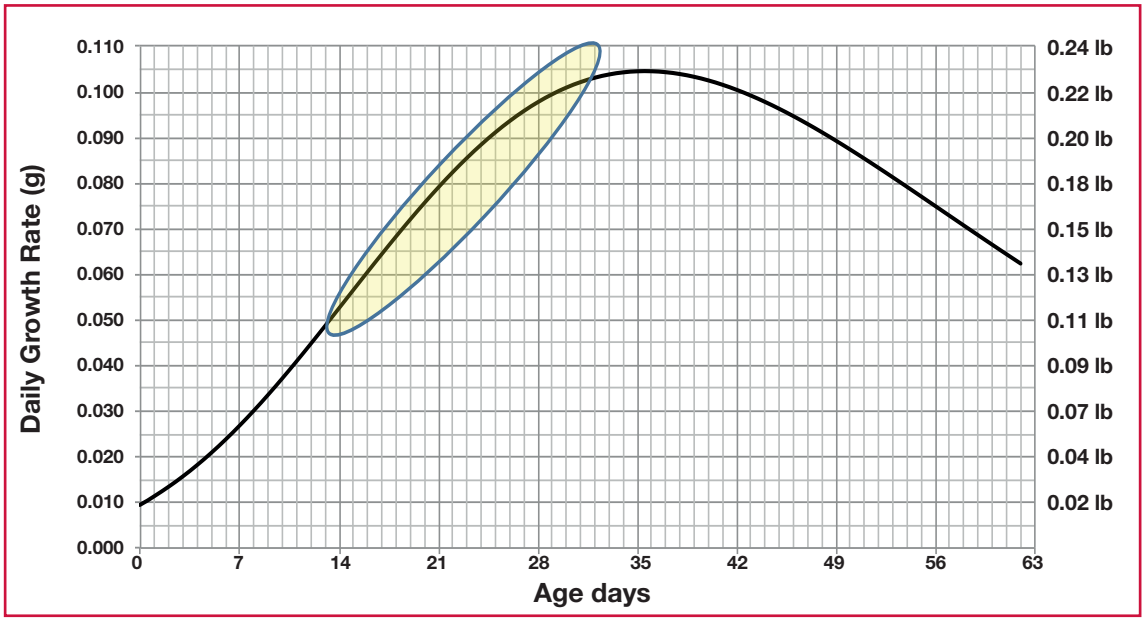
Based on the results of these trials it can be concluded that reductions in dLYS (dLYS deletion) for specific age periods is an effective and practical approach to reduce the incidence and severity of WB and WS. However, this method needs to be implemented properly. In the extreme approaches (25% reduced dLYS fed between 12 and 26 d in a 48 d grow-out period and a 15% reduction in dLYS fed between 12 and 40 d in a 61 d 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 d in a 48 d grow-out period) and 45% (12-40 d in a 61 d 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 d 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 d (**Figure 18**). Maximal broiler growth occurs between 28 and 35 d, 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:

- Age of starting dLYS reduction – not before 14 d.
- 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).
- 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 d; however, bullet point two (above) should be respected to arrive at the appropriate age period of implementation.

Figure 18. 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 42 d:

Cumulative feed at 42 d = 4739 g / 10.4 lbs

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

Feed to 14 d = 537 g / 1.2 lbs

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

8.4. Early growth and BM

The internal Aviagen studies where either feed intake (**Table 3**) or nutrient density (**Table 4**) were only reduced in the first 11 d 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 and SS. The evidence from both research and field data supports the conclusion that poor growth in the first 7-12 d after hatch increases the risk of BM. The goal is to achieve a 7 d body weight that is at least four times the weight of the chick at day-old. For more information on early growth and daily growth rate, please see **Section 13.3**.

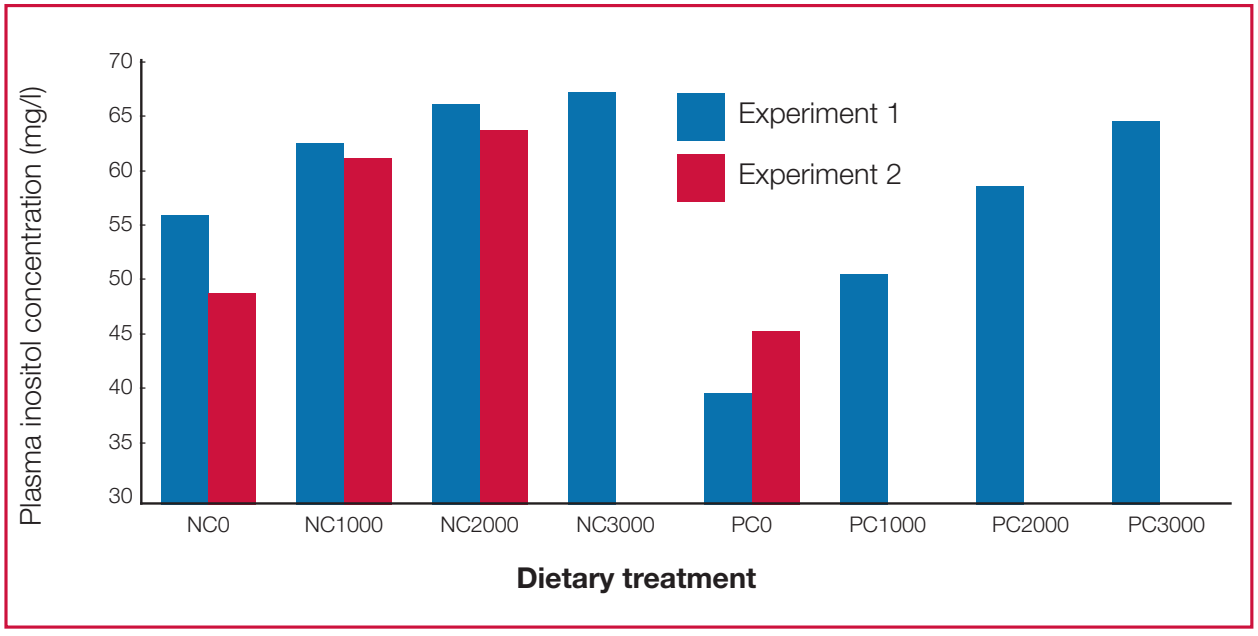
9. Nutrition and BM

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 (2.2 lbs) 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 19**, 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 BM.

Figure 19. 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 [2.2 lbs]) 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 BM, 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 d.

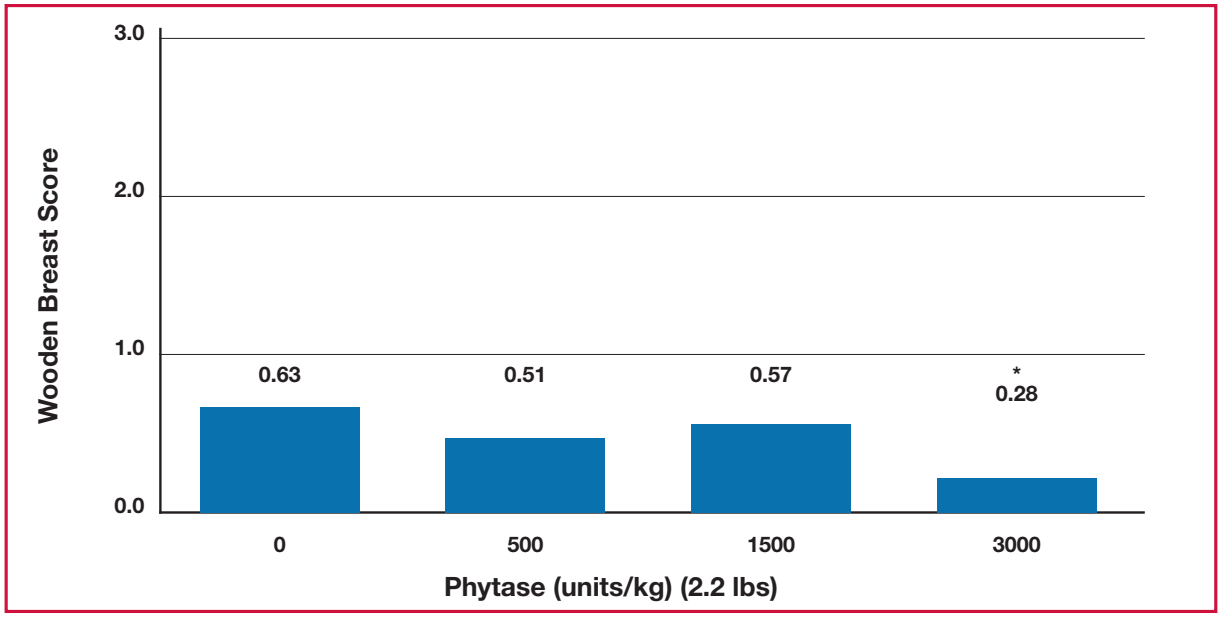
Phytase level	BW kg (lbs)	FCR adj[1]	Carcass Yield[2]	Total white meat yield[2]	Wooden breast score[3]	White striping score[3]
Control (0 FTU)	3.47 (7.65)	1.69[a]	77.89[b]	28.07	1.17	1.05
500 FTU[4]	3.50 (7.72)	1.67[ab]	78.33[a]	28.28	0.99	0.93
1500 FTU	3.54 (7.80)	1.67[ab]	78.28[a]	28.12	1.01	1.01
3000 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 BM. Furthermore, supplementing 3000 phytase units/kg significantly reduced WB score in Ross 708 broilers at 46 d (**Figure 20**).

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 BM, 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 BM.

Figure 20. Wooden breast average score in Ross 708 male broilers at 46 d fed various levels of phytase supplementation.



9.2. Lysine deletion and phytase super-dosing

Given the results obtained from the various lysine deletion (**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 (11-21 d; 11-29 d; 15-25 d; 15-33 d; 19-25 d; 19-39 d) and phytase doses (500 or 1500 phytase units) were fed, and broiler performance and muscle myopathies assessed at 38 d and 48 d. 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 critically 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 (32 d), but this response was inconsistent later in life (39 d or 46 d). Overall results suggest that higher levels of ARG could provide some benefit in lowering muscle myopathies. Corzo et al. (2021) determined the ARG:LYS ratios responses in YPM x Ross 708 males from 25 to 42 d (**Table 7**). Optimal weight gain, FCR, and breast yield at 42 d were estimated at ARG:LYS ratios of 129, 116, and 109, 112 and 109, respectively. Absolute and relative weights of thighs increased linearly with progressive increases in ARG:LYS ratios until 127 without changing breast yields significantly. A tendency to ameliorate muscle myopathy challenges was observed, and it is possible that the shift in carcass traits could have provided nutritional support to normal muscle growth of thigh and breast muscles [unpublished].

Table 7. Growth performance of YPM × Ross 708 male broilers fed diets varying in digestible arginine to digestible lysine ratio from 25 to 42 d.

ARG:LYS ratio	BW Gain (g)	FCR (g:g)	Breast Yield (%)	Thigh (g)	Wooden Breast Score	White Stripe Score
0.79	1602	1.99	26.66	376	0.09	0 .41
0.89	1687	1.93	27.00	392	0.15	0.28
0.99	1712	1.85	27.37	395	0.23	0.39
1.09	1752	1.82	27.77	403	0.26	0.31
1.19	1772	1.83	27.21	412	0.23	0.20
1.29	1784	1.81	27.25	419	0.13	0.27
SEM	12.3	0.010	0.07	1.56	0.045	0.040
Optimum	129	116	109	>129	109	>129

Adapted from Corzo et al., 2021

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 a 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 BM were observed with the different HIS:LYS ratios.

9.5. Methionine+Cysteine (TSAA)

A recent internal trial was carried out studying various dTSAA:dLYS ratio profiles throughout the different feeding phases when fed to Ross 308 AP male broilers. Similarly, to the observations of Corzo et al. (2021), increased dTSAA:dLYS ratios (>78) shifted the carcass compartments yields which resulted in a slight reduction in breast yield, higher leg quarter yield, and consequently lowered wooden breast score.

9.6. Branched Chain Amino Acids (BCAA)

The branched-chain amino acids (BCAAs) leucine (LEU), valine (VAL), and isoleucine (ILE) are essential amino acids and cannot be synthetized by animals. Given the structural similitude among them, antagonisms and interactions have been well documented, particularly when excessive Leucine is provided. The nutritional requirement for digestible LEU is achieved at a ratio to LYS at 110 across all feed phases (Baker and Han,1994; Aviagen, 2022). Commercial feed formulation based on either wheat or corn is rich in LEU which generally exceed the recommendations to levels of 130 or higher. Some regions in which either the availability of soybean meal is inconsistent or costly, or yellow skin pigmentation is required, may observe dLEU:dLYS ratios of up to 170-190 due to excessive use of corn co-products like DDGs and corn gluten meal. Aviagen internal trials have confirmed that increased dLEU:dLYS ratios above 110 requires further adjustments in dVAL and dILE in order to maintain live performance. When higher dLEU:dLYS ratios (>140) coupled with higher dILE:dLYS ratios (>70) enhanced the breast meat yield. It is well known that LEU plays an important role as a potent activator of mTORC1 which consequently affects protein synthesis. On the other hand, such increase in breast yield achieved with elevated ratios of dLEU and dILE to dLYS resulted in a higher incidence of SS. Therefore, this increase in breast muscle deposition proportioning may not be beneficial for breast muscle structural integrity.

9.7. Other Amino Acids

The nutritional requirements of essential amino acids have been well established, and remain under continuous evaluation in order to keep the information current and economically applicable. But considerably less attention has been given to a specific group of amino acids often referred to as non-essential amino acids (NEAA). Despite having received less attention than essential amino acids, NEAA are quantitatively and proportionally as important to the integrity of all bodily tissues. They play important roles in skin integrity, feather development, and tendon and collagen biosynthesis. Collagen is an important component of connective tissue, providing structural integrity to the muscle. Collagen synthesis relies on adequate supply of all NEAA, but particularly proline (PRO), OH-proline, and glycine (GLY). These amino acids are typically sufficient in feeds that include animal by products like meat bone meal, poultry meal, etc. Conversely, commercial feeds have progressively lowered NEAA content over time due to restrictions in the use of animal by-products, when providing all plant protein feeds, and the increasing commercial availability of cost-efficient supplementary amino acids amino acids which have contributed to a reduction in crude protein in broiler diets. With lower or no animal by-product use, the total protein content is also reduced, in turn adding pressure to the dietary

supply of NEAA such as proline, OH-proline and glycine which historically have been neglected in the formulation process and could compromise collagen biosynthesis and consequently the structural integrity of the muscle. Exploratory internal studies have demonstrated positive impact of increased levels of NEAA on performance, content of OH-proline in the muscle, and improved muscle integrity which was reflected by lower occurrence of SS.

9.8. Organic 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 BM (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 39 d and 46 d when feeding organic sources, but overall there was no indication that these more bioavailable forms of minerals led to a decrease in BM.

9.9. 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 WS 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 BM. 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 8**.

Table 8. Live performance and incidence of myopathies in broilers provided various anticoccidial programs at 62 d.

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 d, 0-25 d, or 0-39 d. All these various feeding periods were immediately followed by an ionophore in the feed until 62 d.

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 15 d 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 BM effects observed in the first study. Unlike results from the first trial, there was very little growth curve moderation after 15 d 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 d).

9.10. 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 d of life. The latter trial revealed how BM 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 d of pre-starter; 11-14 d of starter) or a regular starter feed program (0-14 d) 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 14 d 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 BM, improvements to WS were observed at 42 d but not 56 d, and no improvements to WB were seen. There appears to be live performance benefits when using a pre-starter feed, but the impact on BM requires further investigation. 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.

9.11. Antioxidants

A possible factor impacting BM is oxidative stress. While Kuttappan et al. (2012e) showed that supplementing diets using good fat quality with vitamin E up to 400 IU/kg (2.2 lbs) 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 (2.2 lbs)) 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 BM. **Table 9** depicts how the addition of any of the antioxidants, or combination, lowered WB at 49 d, although this effect was not replicated at 62 d. Statistical analysis showed that at 49 d all antioxidants significantly reduced severe (score 3) WB by 38-48% (**Table 10**) 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 9. Live performance and incidence of myopathies in broilers supplemented with different antioxidants at 49 d, 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

Table 10. Effect of different antioxidants on the incidence of severe wooden breast (score 3) in Ross 708 broilers at 49 d. If birds receive feed with oxidized fat/oil, they are more susceptible to oxidative stress and would benefit from the addition of effective antioxidants.

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

9.12. 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 (600 g [1.3 lbs] / MT) and compared with a control feed. There were some slight improvements observed in WB (at 49 d but not at 56 d) 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 BM.

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 BM. In both trials, two farms were used, each with two houses that were fed either a standard diet or diet supplemented with 600 g (1.3 lbs) / ton 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.

9.13. Other products

A commercially marketed product containing betaine, ascorbic acid and unidentified phytogenic compound has been claimed to affect the incidence of BM. It received considerable interest from poultry companies due to the 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 BM, 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 11**, 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 11. Live performance and incidence of myopathies in broilers supplemented with the betaine, ascorbic acid and unidentified phytogenic compound product at 49 d.

	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 processing.
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 BM

10.1. Incubation

The chick embryo develops in well-defined stages over 21 d 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 processing and BM, 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 specialized 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 processing. 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 BM has been limited, although neither did any harm to hatchability or breast muscle growth or quality.

For now, the best incubation advice to minimize BM 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 d weights as a minimum of four times placement weight remains best practice to limit the occurrence of BM.

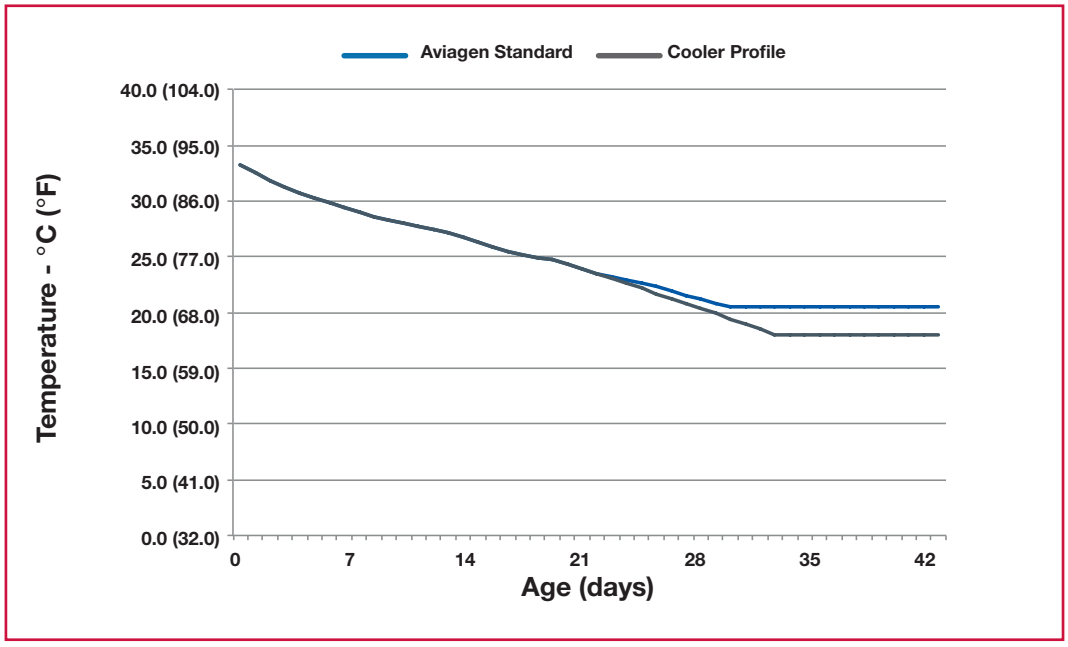
10.3. Environmental/bird body temperature

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 BM were due to an inability of modern broilers to lose heat. They compared a standard temperature profile with a cool profile after 21 d 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 12 d compared to the normal temperature profile declining to 19°C (66°F) but found no difference in the incidence of any BM.

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 BM.

Where BM 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 BM is shown in **Figure 21**. 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 21. Suggested cooler temperature profile to use to reduce BM compared to the standard Aviagen profile. Note that at higher ambient humidities, the temperature may need to be lowered further.



When considering temperature, it is also very important to account for the stocking density as kg of live weight/m^2 (lb of live weight/ft^2). Measured air temperature might not actually reflect the temperature perceived by the birds; the higher the stocking density, the lower the capacity of the broilers to release metabolic heat produced. In general terms, at 30 kg (66.1 lb), the environmental temperature should be around 20°C (68°F), to achieve optimal comfort for the birds. This advice is relevant to the recommended relative humidity levels presented in the ***Parent Stock Handbook***. For every extra kg (lb) per square meter (ft^2), environmental temperature should be reduced by 0.5°C (0.9°F), It is important to note that air speed is vital to remove the heat produced, so air temperature is used as parameter to increase the ventilation rate.

As mentioned above, stocking density is another aspect to consider in order to control BM. Data from the field has shown that higher stocking densities (kg [lb] of meat/m^2 [ft^2]) may increase the risk and severity of BM. Aviagen remains confident in recommended stocking density and temperature advice, but it is important to note that if stocking density is changed to levels other than those recommended, it may be necessary to alter temperature profiles accordingly.

The effect of environmental conditions (inside and outside temperature) and production parameters (number of birds in the house and bird weight) must be considered when calculating bird heat production (W/kg; lb). Determining the amount of heat the birds produce will help to ensure that the house temperature is lowered to a temperature that is appropriate based on bird body weight and stocking density.

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 BM. 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 BM, possibly 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, thereby increasing the risk of muscle cell damage. An analysis of broiler flock data from one processing plant did observe an increase in BM 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 42 d 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 BM 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 BM 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 BM, but the evidence does suggest that light can affect muscle development. Two internal Aviagen studies looked at the effect of light program on the incidence of BM, 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 BM 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 BM 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 DPM can be induced by vigorous wing flapping in both broilers and turkeys (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 processing 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).

Although there is minimal evidence that myopathies such as WB and SS can be induced by excessive bird activity, 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. Flock thinning

Many companies practice thinning, or removing 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 plant's requirement for lower bird weights for particular markets.

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 BM. First, 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. Second, 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 (0.26 lbs) 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 BM

There are no clinical signs of BM 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.

BM can be observed in all breeds/strains of chickens as early as 14 d, with varying prevalence under a wide-range of processing 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 a etiology (**Sections 7.1** and **7.2**). Bilgili (2016) concluded that there is no evidence of infectious and/or pathogenic agents associated with BM.

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

This section was written and reviewed by Dr. Sarge Bilgili, Professor Emeritus Auburn University.

12.1. Effects of bird handling prior to processing

Pre-processing flock management and husbandry systems are very important as they relate to growth rate (muscle tissue), bird activity (metabolic, skeletal and cardiovascular) and consequently in the occurrence of BM. DPM has been directly associated with excessive wing use (to aid in balance, locomotion, cooling, etc.) and activity (fear and escape behavior). Consequently, husbandry practices should focus on limiting wing activity during grow-out, especially close to processing. 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 activities), 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 under heat load 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. DPM has also been associated to underlying gait problems (arthritis, synovitis and tenosynovitis) due to compensatory postural adjustments (i.e., wing walking).

The influence of bird activity on other BM is not well defined, although strains and micro-tears associated with muscular activity can overwhelm the repair processes. Compared to mammals, the inflammatory response is extremely rapid in birds and associated tissue responses can be significant. Bird handling and associated struggle during catching and crating can cause damage when excessive, but usually in the form of wing dislocations, muscle hemorrhages 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 BM and carcass quality. In the event of prolonged fasting periods (voluntary or imposed) the glycogen reserves (in the muscle and liver) are all but depleted, which results in high muscle pH after processing (i.e., DFD meat). Furthermore, prolonged feed withdrawal can cause a change in metabolism resulting in a catabolic state leading to protein degradation and muscle (yield) losses. Recent internal Aviagen data showed that increasing feed withdrawal time by 4 hours increased the incidence of SS. It is important that the recommended feed withdrawal time of between 8-12 h before expected processing is maintained.

12.2. Processing

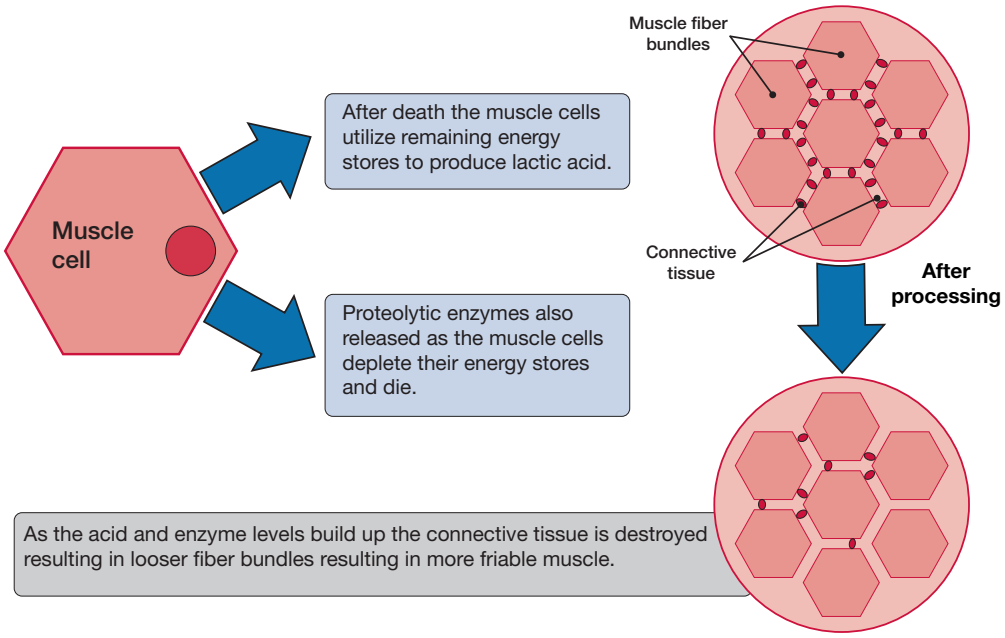
By definition, BM are growth and muscle development associated structural changes. Processes, such as shackling, stunning, bleeding, scalding, and de-feathering can only alter the extent of their physical manifestation. Little or no information is available on the effects of electrical currents (electrical stunning) or modified atmosphere (gas stunning) on BM. The onset of rigor mortis (muscle rigidity) after processing can be accelerated by the stunning and electrical stimulation methods used. Voltage (>200 V), high amperage, pulse frequency and, duration, and location (pre- or post-de-feathering) 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) are typically over-compensated by higher scalding temperatures and de-feathering 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, in a comparison of two processing 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 de-feathering machine, as confirmed by a higher 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 continued metabolic activity of muscles post-mortem. After death the muscle cells continue to breakdown glycogen to produce lactic acid, which reduces the pH and stimulates the proteolytic enzymes to degrade the structural components of the muscle (connective tissue), resulting in a softening of the meat (**Figure 22**).

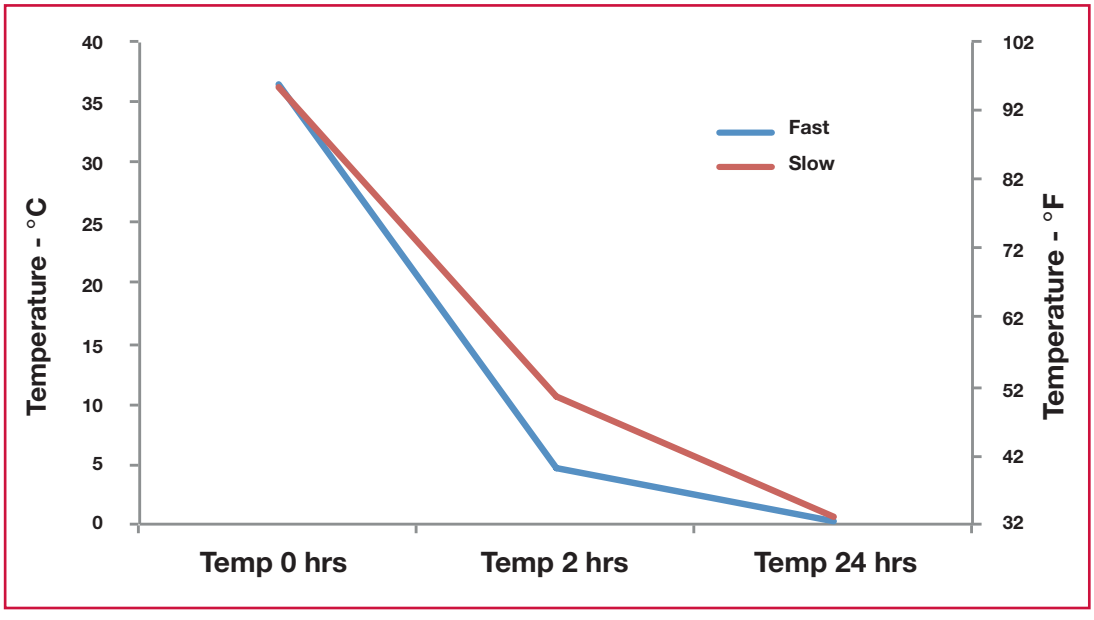
Figure 22. Post-mortem activity in muscle cells.



This degradation by the lactic acid, low pH and proteolytic enzymes only occurs while the meat is warm. Deep muscle temperatures tend to remain high for longer periods during chilling, especially in high breast yield strains processed at heavy weights. Therefore, it is important that the carcass temperature is quickly reduced to reduce proteolytic damage and connective tissue degradation.

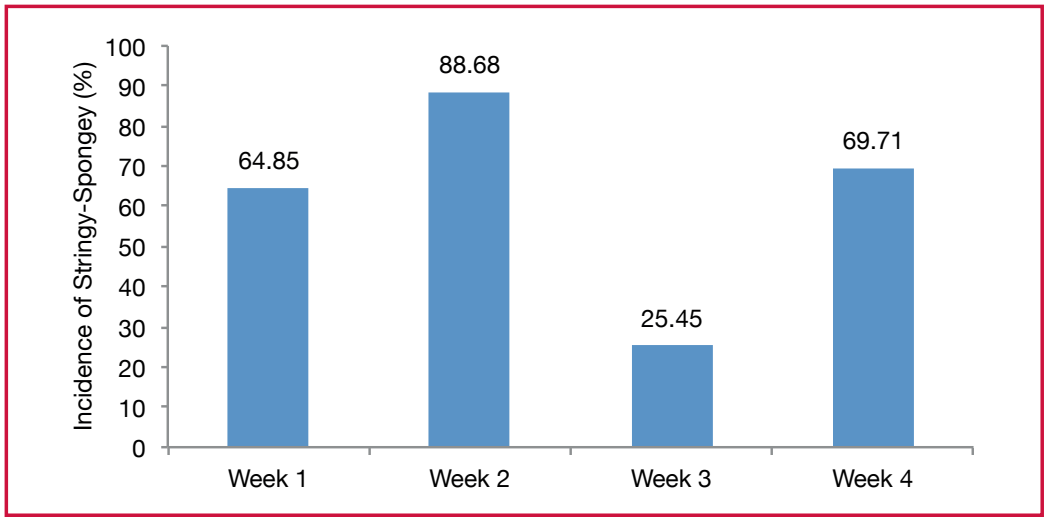
The impact of slower carcass cooling was examined in a trial by Aviagen where two rates of carcass cooling (air chilling) was examined within four consecutive groups of broiler chicken. **Figure 23** shows the mean temperatures obtained for the two cooling planes used for the birds in the trial (each week the birds were randomly split into two groups: one group was fast-chilled and one group was slow-chilled).

Figure 23. Difference in rate of chilling in a trial looking at the effect of chilling rate on incidence of BM.



Following processing and cooling the carcasses were assessed for incidence of BM; the incidence of WS and WB were not affected by the cooling plane, but SS was found to be higher in the birds on the slow-cooling plane relative to their fast-cooled counterparts (**Figure 24**).

Figure 24. Relative increase in incidence of SS 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** 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	23 L:1 d, 27 lux to 7 d 20 L:4 d, 11 lux 8-48 d
Diets	Starter (1-12 d, crumble) Grower (13-31 d, pellet) Finisher (32-40 d, pellet) Withdrawal (41-48 d, 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 48 d Processing: 32 and 49 d 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	0-62 d
Lighting	23L:1 d, 25 lux to 7 d 18L:6 d, 10 lux 8-48 d
Diets	Starter (1-11 d, crumble) Grower (12-28 d, pellet) Finisher 1 (29-40 d, pellet) Finisher 2 (41-48 d, pellet) Withdrawal (49-62 d; 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)

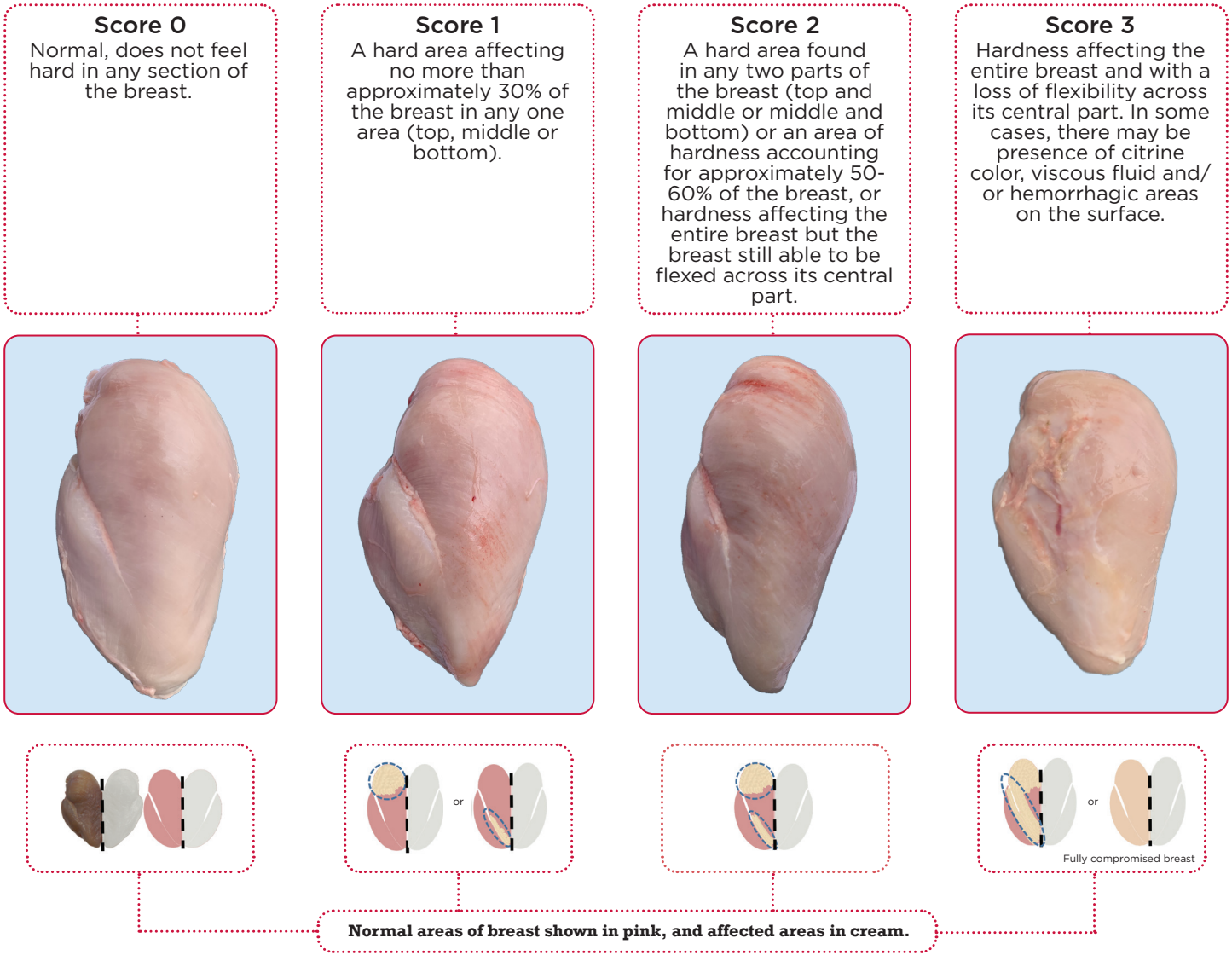
Treatment	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Last day fed	11	28	40	48	62
1	Control	Control	Control	Control	Control
2	Low	Control	Control	Control	Control
3	Low	Low	Control	Control	Control
4	Low	Low	Low	Control	Control
5	Low	Low	Low	Low	Control
6	Control	Low	Control	Control	Control
7	Control	Low	Low	Control	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.

13.2. Breast Myopathy scoring

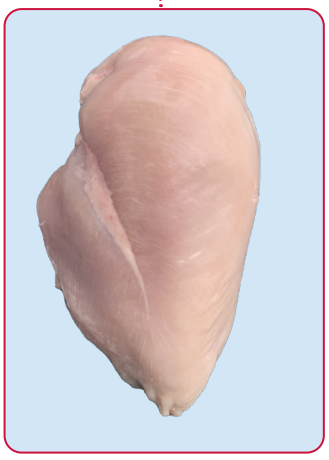
The breast myopathy score cards below give an indication as to the location and severity of some of the previously mentioned myopathies. *The scoring system shown on these cards is for research and investigation purposes only: the scores do not show breast meat sorting criteria in the processing plant.*

Wooden Breast

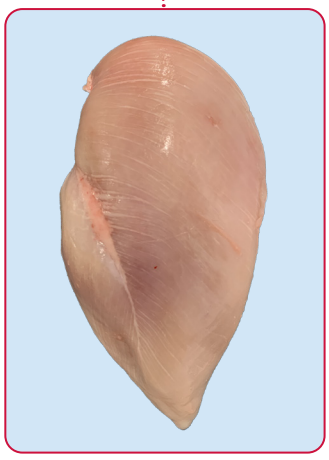


White Striping

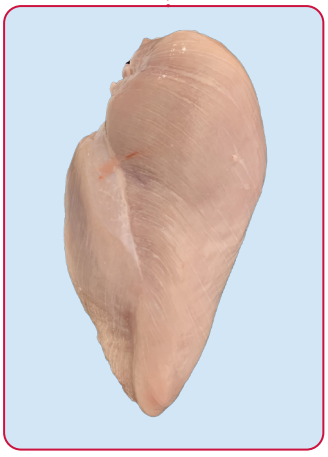
Score 0
Normal, no striping on any section of the breast.



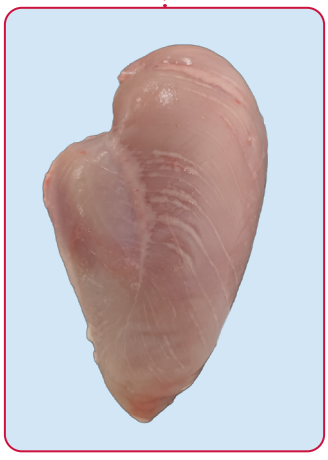
Score 1
Noticeable striping covering approximately 30% of the breast surface.



Score 2
Noticeable thin striping (<2mm/0.08 in) covering the entire surface of the breast, or some thicker lines (3-4mm/ 0.12-0.16 in) covering approximately 40% of the breast.

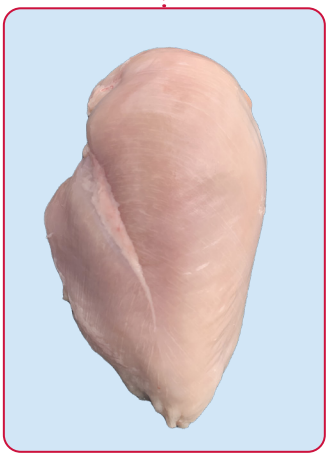


Score 3
Extensive coverage of the breast with a mixture of thick (3-4mm/0.12-0.16 in) and thin lines (<2mm/0.08 in).

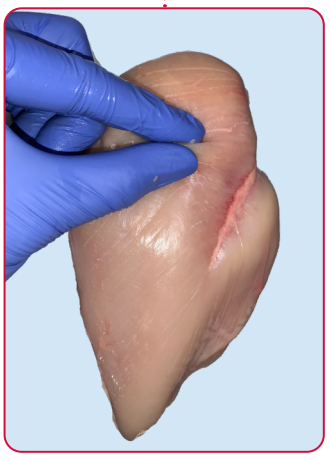


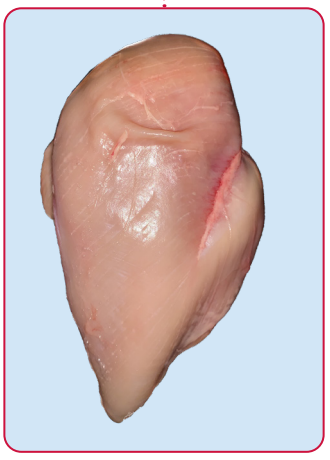
Stringy-Spongy

Score 0
Normal breast with no sign of stringy-spongy.

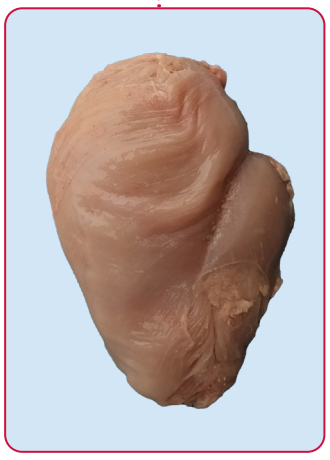


Score 1
An area of open breast fibers affecting no more than approximately 30% of the breast in any one area (top, middle or bottom), or an area of looseness that can be pinched and lifted accounting for approximately 40-50% of the breast meat.

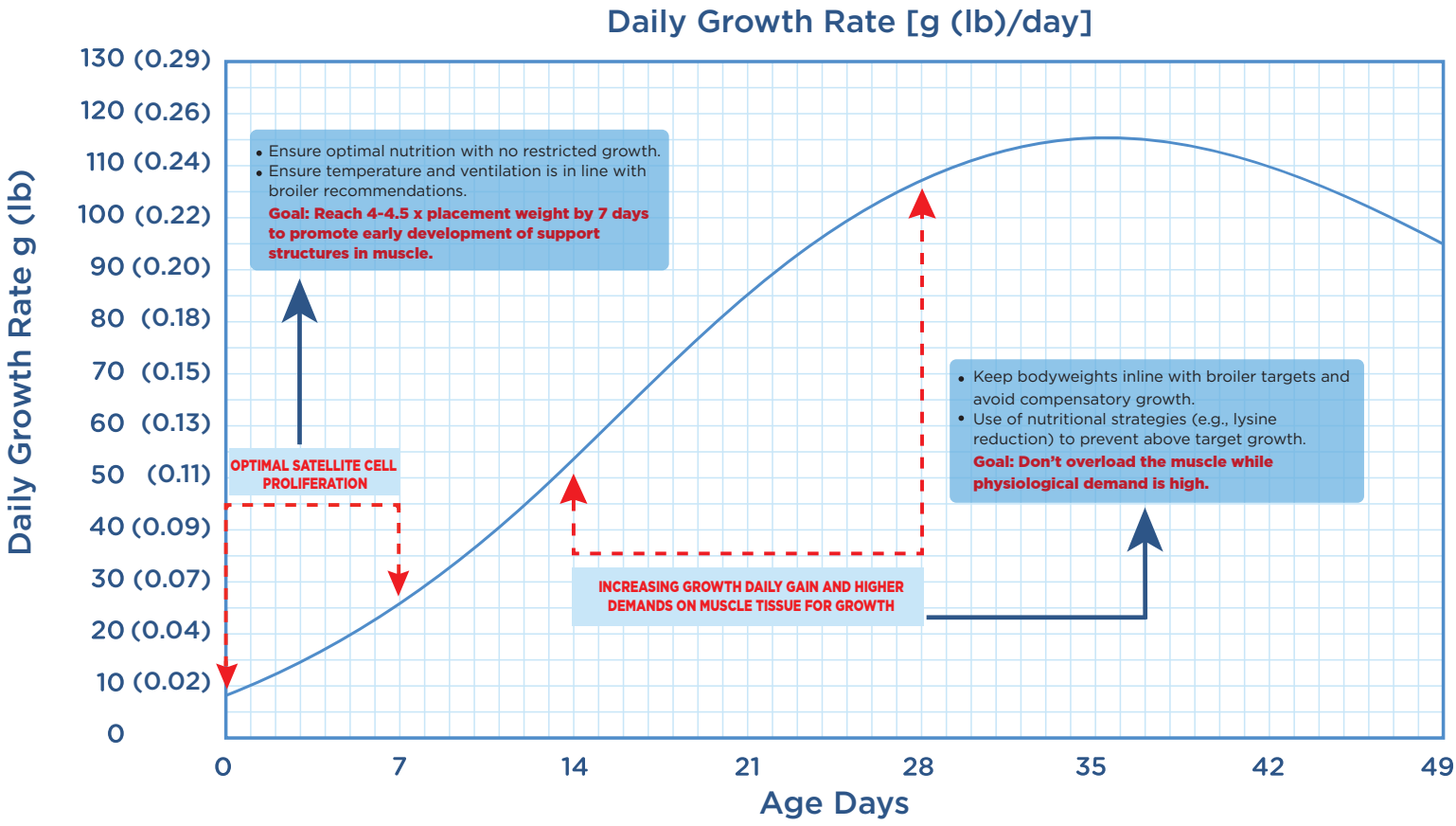




Score 2
Two areas of breast showing open areas with exposed fibers, or one area showing severe damage with exposed fibers affecting >50% of the surface.



13.3. Daily growth rate



Ensure optimal incubation conditions to prevent hypoxia & overheating, which can inhibit muscle embryogenesis.

KEY POINTS THROUGH LIFE OF STOCK

- Ensure the birds have adequate ventilation to provide oxygen and allow heat to dissipate.
- When necessary use cooler temperature profiles to prevent overheating.
- Ensure the birds have adequate levels of antioxidants where required.
- Ensure proper amino acid balance, especially if on all plant based diets.

PROCESSING

- Avoid lactic acid build up during transport and in lairage by ventilating birds properly.
- Ensure that scalding temperatures are not too high, as this can overheat the muscle causing damage.
- Avoid excessive de-feathering, as this can damage the meat (e.g., this may be a concern when low scald temperatures are used).
- Ensure optimal cooling of carcasses after death to prevent post-mortem build-up of lactic acid in the muscle, which can destroy connective tissue.
- The use of compounds such as peracetic acid may impact connective tissue and should not be overused.

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