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**Factors affecting the production of poultry meat for processing**

**Nicholas Andrew Hover**

A handwritten signature in black ink, consisting of a stylized 'N' followed by a cursive 'A' and 'H', with a horizontal line underneath.

**A thesis submitted for the degree of  
Doctor of Philosophy  
at the  
University of Glasgow**

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Scottish Agricultural College Ayr  
July 2003**

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## Abstract

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The link between poultry animal production factors and poultry meat processing has not been studied in detail. The effect of factors such as genotype, sex, age, diet and muscle type on meat processing capabilities such as post-mortem pH, soaked, cooked yield, texture and binding of whole and comminuted meat are reported here. To determine the processing capabilities of the meat various methods were employed including muscle fibre typing, microscopy and electrophoresis.

The breast muscle was more suited to processing than the thigh, particularly with regards to texture. Genotype was the most significant production factor for processing capabilities: broiler-type birds produced meat which gave higher soaked and cooked yields, was less tough, and improved meat bind. Generally, older animals produced meat less suitable for processing. Diet had little effect on the processing capability of the meat.

The addition of salt and phosphates to poultry meat had a more significant effect on processing behaviour than any animal production factor. Phosphate increased the soaked and cooked yield, reduced toughness and increased meat bind. Salt improved the processing performance more than phosphate. Salt and phosphates added together improved processing performance more than either alone. This synergistic result was independent of animal production factors.

These findings indicate that muscle location, genotype and bird age are the most significant animal production factors. Additionally, salt and phosphate affect the processing capability of meat more than any of the animal production factors. Muscle fibre type has no effect on meat processing capability. Microscopic observations suggest that protein dispersion, decrease in porosity and void size increased cooked yield and meat binding. The electrophoresis results showed that actin and myosin are the two proteins most dispersed by the action of salt and phosphate.

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## **Declaration**

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I declare that this thesis and the work presented in it are my own. All assistance received has been acknowledged.

Nicholas Andrew Hover

July 2003

## Publications

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N.HOVER, D. LEWIS & WOODS, B. (2002) Novel fibre typing technique for meat quality assessment. *British Poultry Science*, **43**: 56-57.

N.HOVER, A.J. GAVIN, D. LEWIS & R.M. MCDEVITT. (2002) Effects of live bird and brine treatments on added water holding capacity of breast meat. *British Poultry Science*, **43**: 55-56.

N.HOVER. (2003) Microstructure of broiler breast emulsions. **Meat Science** submitted



# Chapter 1

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## Introduction

Poultry research has centered on the genetic selection and control of nutritional and rearing factors that largely contribute to muscle growth and body conformation. Over the past 50 years the body weight of a 6 week-old meat type chicken has increased from 1.0 kg to nearly 3.0 kg. Likewise broiler breast yield has increased from 11-12 % to 19 % within a 30 year period. One reason for this has been the increase in poultry consumption. When compared to the growth in consumption rate for pork (2.5 %) and beef (0.9 %), poultry has had a growth of 8.3 % (Mandava and Hoogenkamp, 1999). Much of this increase is due to the consumer's perception of attributes such as flavour, texture, taste and white colour. Chicken represents a protein source accepted by all world cultures and religions. In addition to the market change in favour of poultry there has been a change in the type of product purchased, with consumers increasingly choosing to buy processed products rather than whole muscle products. In the UK the total volume of further processed chicken has shown an increase from 92,410 tonnes in 1995 to 105,901 in 1996, with an annual increase of around 14.6 % (Mandava and Hoogenkamp, 1999). The market growth has so far allowed processed products to be manufactured in a fairly empirical way since the market has allowed reasonable margins. However, increasingly the market is requiring manufacturers to optimise their processes in order to retain margins and a more scientific underpinning of the manufacturing operation is required. In particular the linkage between production parameters and processing functionality is poorly understood.

As production of broiler meat has become more cost efficient, the market for 'spent hens' (laying hens that no longer produce a sufficient yield of eggs) has declined to the point where they are a commercial liability to the egg-producing sector. In the UK over 39.24 million boiling fowl (spent hens) are slaughtered annually (DEFRA, 2003). Many poultry processing units cannot take spent hens and this leads to considerable logistical problems for egg producers in addition to the cost of disposing of laying hens.

This meat could be used as pet food or sold to retort canning operations for processing as solid particles in liquid products such as soups, sauces, stews, and gravies or sold as stewing hens. Alternatively, the meat could be comminuted and restructured in the presence of salt and phosphates. Traditionally sausages and other comminuted products are manufactured to use low value cuts of meat and similar commercial products could be used with spent hen meat.

Research has shown that the functional properties of meat can be related to either animal production factors (age, genotype, sex, diet and muscle fibre type) or processing factors (meat pH, comminution and addition of salts and phosphates). These factors will influence the mechanisms which intervene when muscle becomes meat or the chemical composition of muscle, its structure and yield. Yet comparatively little research has been carried out on how these factors affect a processed and comminuted poultry product.

The purpose of this chapter is to review the available knowledge concerning the structure of meat, animal factors and how these affect the functional properties of muscle proteins in processed poultry products. The intention is not to produce a fully comprehensive account of the data relating to meat structure and composition but to highlight those developments that have particular relevance to avian meat science. The approach taken is an integrative one highlighting those factors that are related to processed and comminuted products.

## **1.1 Meat technology**

### *1.1.1 Structural aspects*

A structural approach greatly clarifies which components of meat are responsible for its tenderness, water-holding capacity and appearance, and the events during processing (Offer *et al*, 1989a). Structural investigations on meat are also very helpful since establishing the spatial arrangement of components can identify the components responsible for meat quality and greatly clarify the mechanism responsible.

Visible differences between poultry meat and that of red meat animals are apparent. Despite these differences, the striated muscle of mammals shares a common organisation with that of poultry in having the striated contractile cells held in alignment by a connective tissue framework.

Every muscle is connected to a tendon. Surrounding each muscle is a thick connective tissue sheath, the epimysium, which is continuous with the tendon. The muscle is divided into muscle fibre bundles by the perimysial connective tissue network (Figure 1). The coarse or primary perimysial network, which is visible to the naked eye, is further subdivided by thinner sheets of perimysial connective tissue, typically defining a bundle of muscle fibres of the order of 1 mm across. The collagen fibres in the perimysium are crimped and arranged in a criss-cross lattice (Rowe, 1974). In muscle at rest length, the collagen fibres in the perimysium are arranged at an angle of about  $55^\circ$  to the muscle fibre axis but assume larger angles as the muscle shortens (Rowe, 1974). Individual muscle fibres are separated from one another by the endomysial connective tissue network.

Under phase contrast microscopy, relaxed muscle has dark bands of dense protein structure which are anisotropic under polarized light and as such have been termed A-bands (Figure 2). The I-bands derive their name from the isotropic refractiveness of the lighter bands in which the constituent proteins are less dense. In the central region of each A-band is the less dense H-zone which itself is bisected by the dark M-line (Figure 3 and Figure 4). The refractive Z-line is also clearly visible as a sharp dark band within the middle of each I-band (Gault, 1992). Electron microscopy has shown that this banded appearance is due to the extent of overlap of two sets of filaments.

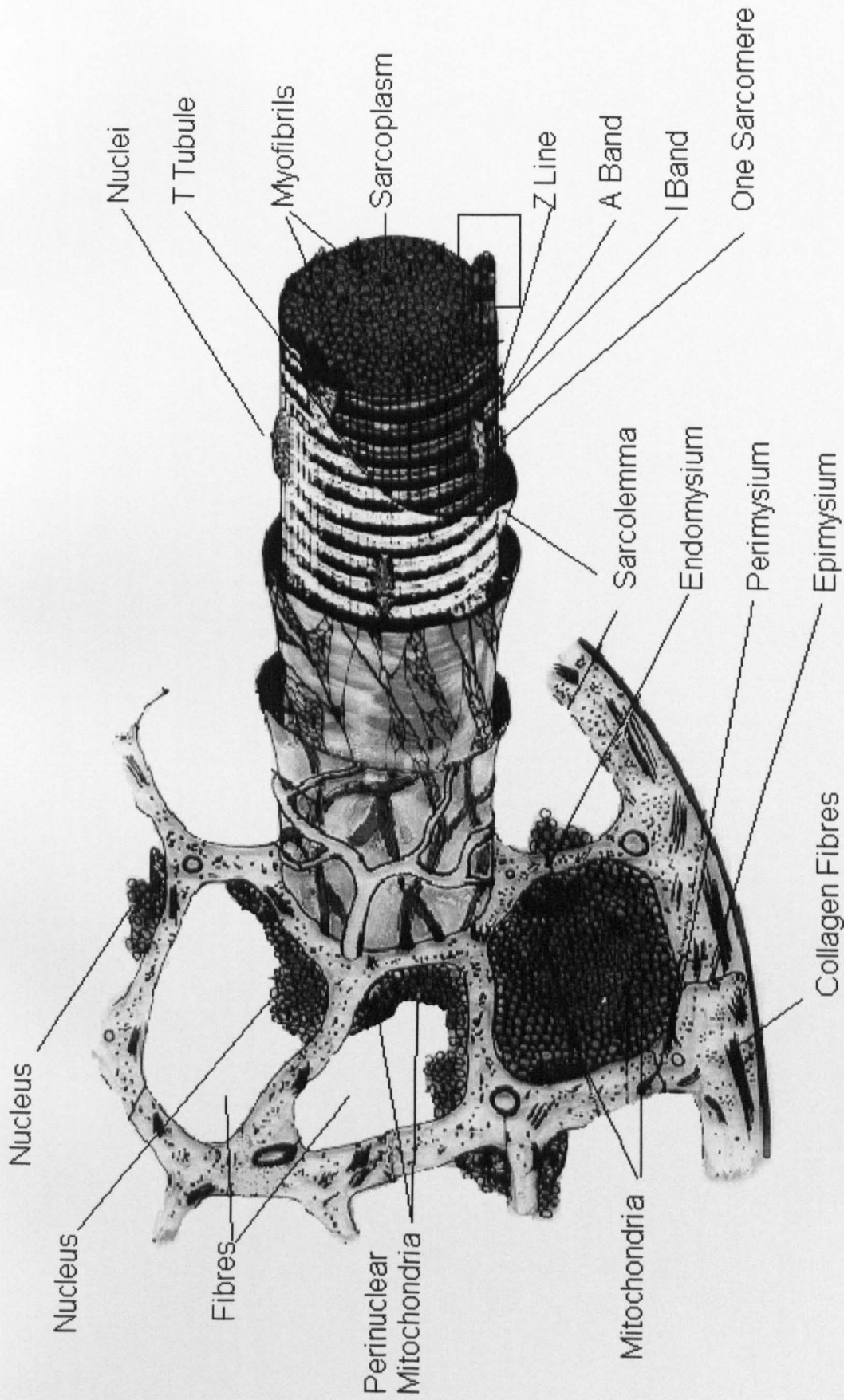


Figure 1 Muscle fibre and associated structures (C.S.I.R.O, 1971)

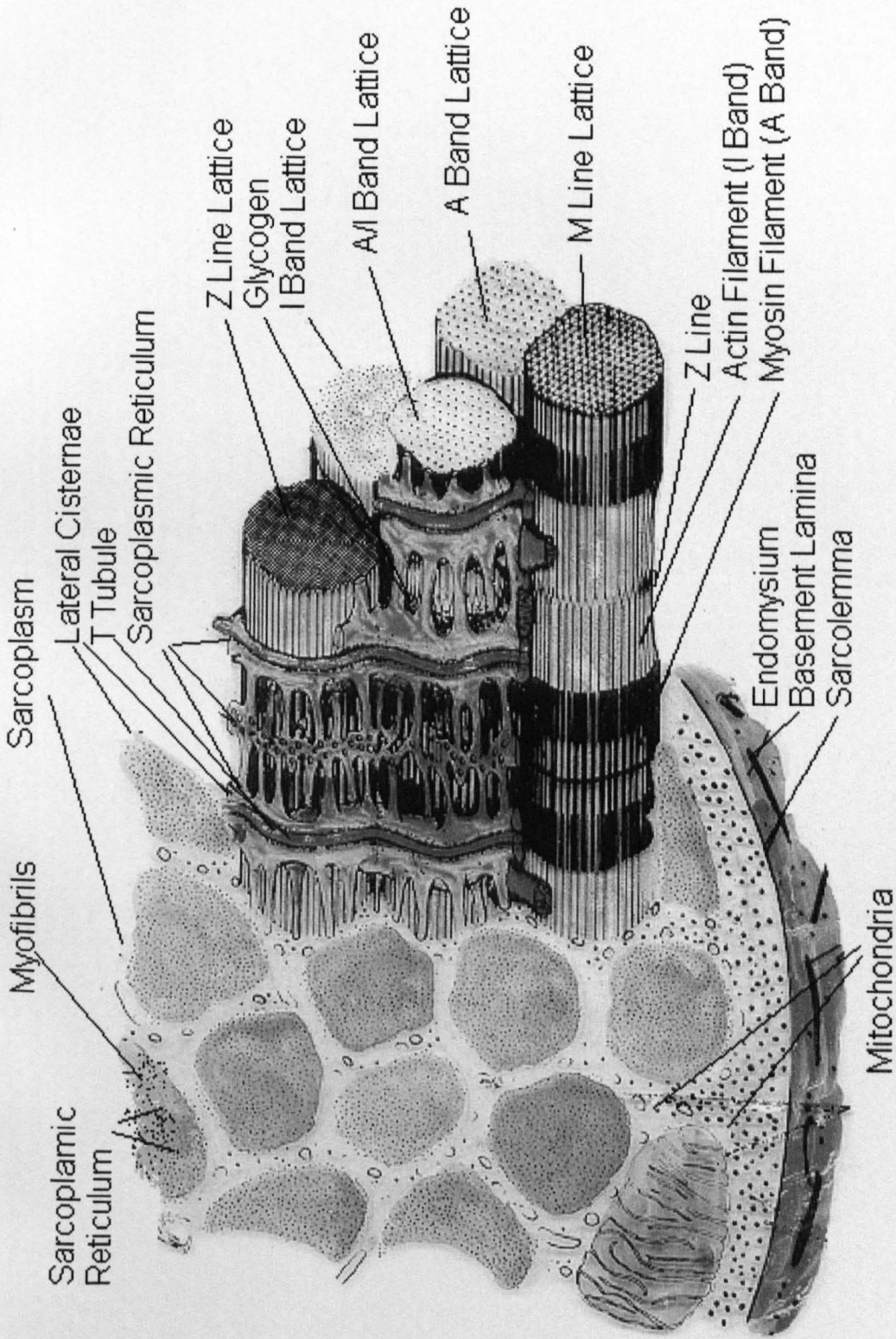
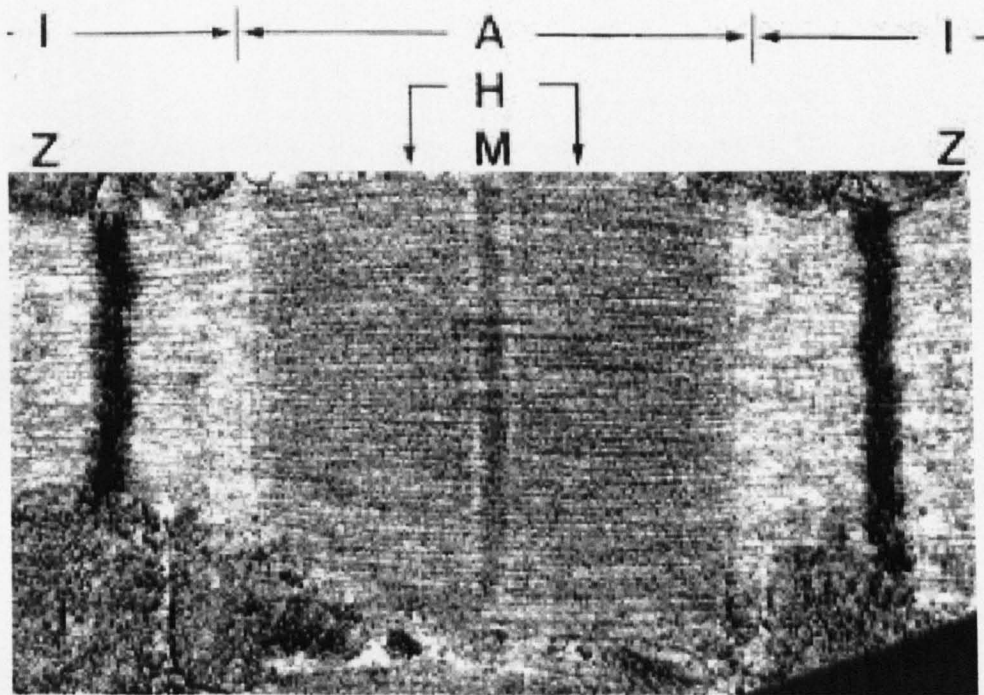
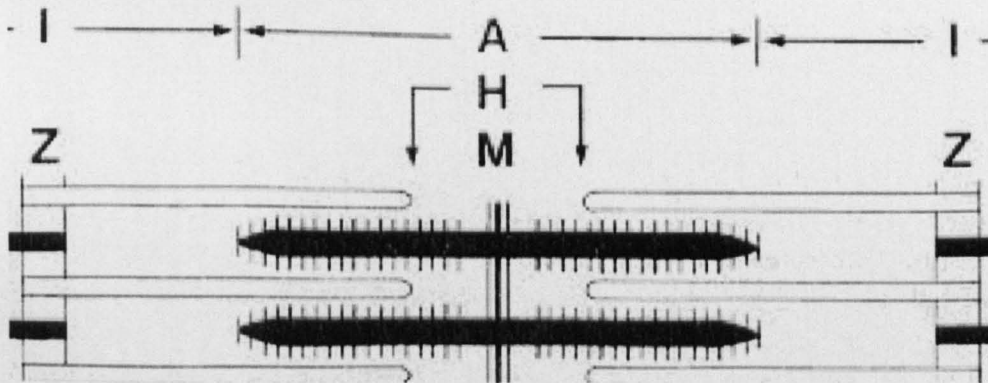


Figure 2 Enlargement of muscle fibre structure (C.S.I.R.O, 1971)



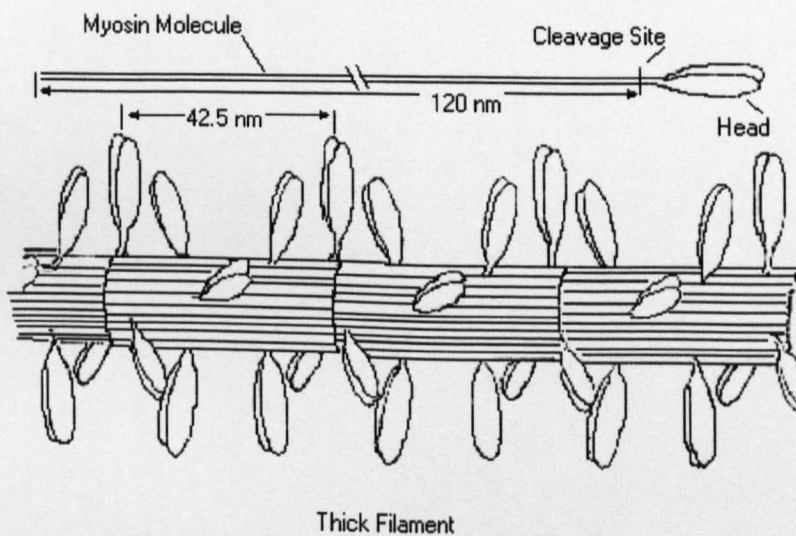
**Figure 3** Electron micrograph of a longitudinal section through skeletal muscle (Neuromuscular, 2002).



**Figure 4** Schematic of skeletal muscle sarcomere, (I= I band, A= A band, H= H zone, Z= Z line, M= M line) (Neuromuscular, 2002).

### 1.1.2 Thick filaments

The thick filaments, (Figure 5), are formed by the association of up to 400 individual molecules of the filamentous protein myosin (Harrington, 1981). The myosin molecule is a 480,000 dalton protein characterised by having two globular head sections attached to a long tail (Slayter and Lowey, 1967; Elliott and Offer, 1978). At the quaternary level, the molecule is composed of two large sub-units (the heavy chains) each having a molecular weight of *ca.* 200,000, and four smaller sub-units (the lighter chains) of variable molecular weight in the region of 20,000 (Lowey and Holt, 1972; Weeds, 1976).



**Figure 5** Schematic of a skeletal thick filament adapted from (Neuromuscular, 2002).

Both heavy chains are arranged as  $\alpha$ -helices along about 50 % of their length and coiled around each other in a rope-like manner to form the tail portion of the myosin molecule. The remainder of each heavy chain folds separately into the globular head regions of the myosin molecule. Each of these head regions binds two of the light chains. Consequently, the myosin molecule is *ca.* 140  $\mu\text{m}$  long with each head region *ca.* 9 nm in diameter at its widest point. Electron microscopy and low-angle X-ray diffraction studies in the late 1960s gave some insight into the manner in which the myosin molecules assemble to form thick filaments. On each side of the central clear zone of the A-band, all the myosin

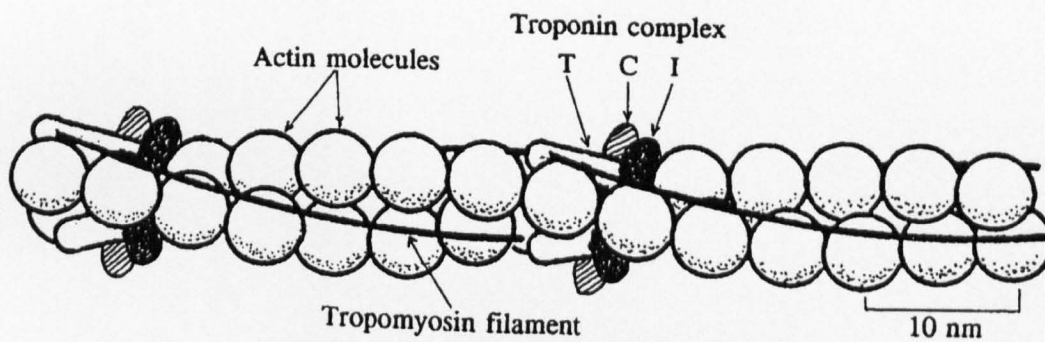
molecules are symmetrically oriented with their heads pointing away from the centre of the filament (Luther *et al*, 1981). This bipolar structural arrangement is responsible for the smooth central zone (150-200 nm) of the thick filaments which consists of the tail segments of overlapping myosin molecules pointing in opposite directions. The ordered packing arrangement of the myosin molecules also accounts for the manner in which three or four pairs of myosin heads protrude from the circumference of the thick filaments at 14.3 nm intervals tracing a helical pathway with a repeat distance of 42.9 nm per helical twist (Offer *et al*, 1989b). The myosin heads have ATPase activity (Squire, 1997; Holmes and Geeves, 2000) which plays a role in muscle contraction, force and movement are produced through a conformational change in myosin heads.

At least seven other proteins are associated with the thick filaments. Immunofluorescence studies have shown the presence of M-protein, myomesin, and the enzyme creatine kinase in close association with the M-line (Turner *et al*, 1973; Masaki and Takaiti, 1974; Trinick and Lowey, 1977). While myomesin is found in all striated muscle fibres, M-protein is restricted to fast skeletal and cardiac muscle fibres suggesting that myomesin is involved in the general control of A-band structure and M-protein is required to accommodate greater stress in fast muscle fibres (Grove *et al*, 1984; Carlsson *et al*, 1990). C-protein, which appears as several regularly spaced stripes along the length of the thick filaments on either side of the M-line, is closely associated with the tail segments of the myosin molecules and may have a structural role in controlling the formation of the thick filaments (Offer and Moos, 1973). No structural role has yet been established for the minor F- and H-proteins of the A-band, (Miyahara *et al*, 1980; Yamamoto, 1984), while the I-protein, localised near each end of the A-band, is known to inhibit myosin activity (Ohashi and Maruyama, 1985; Chou *et al*, 1996; Sheard *et al*, 1999).



### 1.1.3 Thin filaments

Thin actin filaments, (Figure 6), 8 nm in diameter and 1.0 $\mu$ m in length, extend from either side of the Z-line and make up the I-band. The thin filaments are formed when the spherical monomers of G-actin, 5.5 nm in diameter and with a molecular weight of 42,000, condense to form the double-helical filament known as F-actin. The two actin strands, which make up each thin filament, twist across each other every 36.5 nm, there being about 13 G-actin monomers within each of these repeating sections of the filament (Hanson and Lowy, 1963; Ohashi and Maruyama, 1985; Squire, 1997).



**Figure 6** Schematic of a skeletal thin filament (Neuromuscular, 2002).

The other major proteins of the thin filaments are the regulatory proteins tropomyosin and the troponin complex (Ebashi and Kodama, 1999; Tobacman and Butters, 2000). Tropomyosin is a thin filamentous protein, 41 nm in length, and composed of two  $\alpha$ -helical polypeptide chains coiled around each other to give the molecule a rope-like appearance. It forms tightly bound end to end aggregates which run the entire length of the F-actin filament along each of its helical grooves (Hartshorne and Mueller, 1968; Ebashi and Endo, 1969; Lehman, 2000). The globular troponin complex, composed of the three sub-units troponin T, C and I confer calcium sensitivity on the filament (Perry, 1998; Zhou *et al*, 2000). Troponin is found attached to each tropomyosin molecule at 38.5 nm intervals along both sides of the F-actin double helix (Hartshorne and Mueller,

1968; Zhou *et al*, 2000). The minor proteins are  $\beta$ -actinin (Maruyama *et al*, 1977) which caps the free ends of the thin filaments,  $\gamma$ -actinin, (Kuroda and Maruyama, 1976), known to inhibit actin polymerisation, and more recently discovered paratropomyosin (Takahasi *et al*, 1985) located at the A-I band junction.

#### 1.1.4 Cytoskeletal framework

Muscle cells contain cytoskeletal elements, which form a continuous intracellular network spanning the entire muscle length. Titin, the third most abundant protein in muscle after myosin and actin, was first identified by Maruyama. *et al*, (1976). It is characterised by its very high molecular weight, 2400 to 3000 kDa, and elastic properties; (Maruyama *et al*, 1984; Wang *et al*, 1984). A single titin molecule spans half of the sarcomere having its N-terminus associated with the Z-line and its C-terminus to the M-line. In the native form, titin exists in the  $\alpha$ -form, however, only the properties of  $\beta$ -titin, produced by the proteolysis of  $\alpha$ -titin, have been studied in detail (Wang *et al*, 1984; Trinick *et al*, 1984). Wang *et al* (1984) suggest that titin can be stretched up to 1.1  $\mu\text{m}$  long in the relaxed state and is capable of being stretched to 3.0  $\mu\text{m}$ . suggesting that it has some sort of regulatory ability on the other myofibrillar proteins.

Immunoelectron microscopy with 11 monoclonal antibodies, recognising distinct and non-repetitive epitopes, shows that the titin molecule extends from the Z band into the M band (Fürst *et al*, 1989), which coincides with striations of the A band known to harbour two myosin-associated proteins, the C protein and the 86 kDa protein (Craig and Offer, 1976; Sjöström and Squire, 1977; Fürst *et al*, 1989). Labeit (1991) showed that titin consists almost entirely of repetitions of two types of motifs, called class I and class II. Both are roughly 100 residues in length and are related to type III fibronectin and C2 immunoglobulin domains, respectively. Purified titin II molecules have a narrow length distribution of about 900 nm and lack the Z band anchoring domain. Individual titin molecules appear as long strings carrying a single globular head, which seem connected

to the two M-band proteins of apparent molecular mass of 190 and 165 kDa (Nave *et al*, 1989). This repetitive display of 100 residue domains fits the 4 nm beaded appearance seen in electron micrographs of titin (Trinick *et al*, 1984). The 42 to 43 nm repeat observed particularly in the A band (Fürst *et al*, 1989) seems to reflect an 11 domain repeat pattern (Labeit, 1991) and biochemical results prove the interaction of titin with LMM part of myosin and with C protein (Labeit, 1991; Fürst *et al*, 1992). Several sarcoplasmic proteins, including  $\alpha$ -actinin, actin, myosin, C-protein and myomersin, have been identified as titin binding partners (Soterioum *et al*, 1993; Tanabe *et al*, 1994; Sorimachi, 1995; Houmeida *et al*, 1995; Gregorio, 1998; Mues *et al*, 1998). Suggestions have been found in literature that changes in titin are accompanied by changes in tenderness and water holding capacity in meat (Huff-Lonergan *et al*, 1995; Taylor and Koohmaraie, 1998).

Evidence by Locker (1984) supports the theory of 'gap filaments' observed when muscle fibres are stretched beyond the natural overlap length of the thick and thin filaments. Wang and Williamson (1980) identified a protein called nebulin, found in the N-lines, which can represent as much as 5 % of the total myofibrillar proteins. These transversal intermediate filaments spread out from one side of the cell to the other and connect myofibril bundles to the sarcolemma by costameres (Pierobon-Bormioli, 1981). Costameres are comprised of actin binding proteins including vinculin, talin, filamin,  $\alpha$ -actinin and dystrophin. The importance of these structures is that they form the link from the intracellular to the basal lamina and connective tissue. Little is know about these structures. Nethertheless, from microscopic observations it is clear that most myofibrillar structures including the I-band, A-band and Z-line, are perfectly aligned between adjacent fibres, suggesting that a filamentous adjacent structure was branching the extracellular matrix crosslinking them together (Ouail, 1999).

Locker (1984), Wang *et al* (1984), Maruyama (1985), and Wang and Wright (1988) all suggest that gap filaments are seen to be as playing an important part in meat tenderness, although the actual mechanisms are still unclear. The degradation of titin has been

implicated as being responsible for the increasing meat tenderness that occurs during conditioning. On the other hand, King *et al*, (1981) and King (1984), studying mutton, have denied any significance of the degradation of titin in meat tenderness since the protein is destroyed by cooking. Although no disappearance of titin from chicken muscle and no significant differences in the content and electrophoretic pattern of titin isolated from chicken muscle during conditioning were reported by Locker (1984); Fritz *et al* (1993) or Suzuki *et al* (2001).

#### 1.1.5 Stromal proteins

Stromal proteins include collagen, elastin and lipoproteins of the cell membrane (plasma membrane, sarcoplasmic reticulum and mitochondria). Collagen is the main stromal protein, surrounding the whole muscle (epimysium), fibre bundles (perimysium), and individual fibres (endomysium). Several different types of collagen are present in muscle with the epimysium containing predominantly type I, the perimysium types I and III, and the endomysium types IV and V; (Bailey, 1984; Sims and Bailey, 1992). The basic collagen molecule is made up of three  $\alpha$ -helical protein chains wound together. Type I molecules consists of two identical chains, designated  $\alpha 1$  (I), and a third designated  $\alpha 2$  (II); type III molecules consist of three identical  $\alpha 1$  (III) chains. Hydroxyproline side chains contribute to the stability of the triple helix.

Assembly of collagen molecules into fibrils is thought to be governed by the distribution of polar and hydrophobic side chains. Electron microscopy revealed that molecules are axially staggered by a distance of 67 nm or 234 residues (Meek *et al*, 1979). The axial stagger between adjacent molecules enables inter-molecular Schiff based crosslinks to form between an aldehyde group in a telopeptide and a lysine or hydroxyline side chain in a triple helical region. As an animal matures, the Schiff base crosslinks decrease in number and are thought to convert to more stable but as yet unidentified structures. These changes contribute to the lower solubility and higher tensile strength of connective tissue

in older animals. Although the amount of collagen in an animal may increase as animals become older, and this may have an effect on meat toughness, rapid growth of muscle fibres may dilute the relative amounts of collagen in meat. This may be particularly important when measuring the texture of broiler type birds. Still, there is an absence of overwhelming evidence from taste panel studies connecting collagen (I and III) to tenderness. Collagen concentration may be the primary determinant of eating quality, while collagen solubility may determine shear strength measured mechanically (Young and Braggins, 1993).

## **1.2 Further processing**

### *1.2.1 Protein Functionality*

Originally Kinsella (1976) defined protein functionality as any physicochemical property which affects the processing and behaviour of proteins in food systems as judged by the quality attributes of the final product. Dickinson and McClements, (1996) defined functionality as the behaviour features of a food identifiable by human senses as relevant to quality. Processing conditions, environmental factors (pH, ionic strength) and interactions with other components affect the functional behaviour of proteins in food systems (Kinsella, 1976). In further processed poultry products, water binding and water holding capacity, fat binding, solubility, viscosity and emulsification, are the principle functional properties required in raw meat products (Smith, 1988).

### *1.2.2 Water holding capacity*

Much early work considering water holding capacity on pigs centered on the relationship between muscle fibre type distribution and porcine stress-susceptibility (PSS) and pale, soft and exudative (PSE) conditions in pigs was reviewed by Swatland (1983). One problem in reviewing these results is that at the time the halothane gene, now known to influence both PSS and PSE, was present, which may have caused considerable variation

both in quality aspects and muscle fibre type (Klont *et al*, 1998). Thus, a direct comparison between recent and much of the early work is impossible.

Myofibrillar proteins are responsible for the retention of water (about 75 %) in the muscular tissue. A water to total protein ratio of 3.3 to 3.6 and a water to myofibrillar protein ratio of about 5 exists in the lean tissue (Honikel and Hamm, 1994). Water holding capacity is sometimes defined as the ability of meat to retain its own water under external influences such as centrifugation. Water-binding capacity may be defined as the ability of the meat to bind extra water added to it with water absorption or gelling capacity being defined as the ability of meat to absorb water spontaneously form an aqueous environment (Swatland, 1995). It is generally accepted that the water in meat is bound in different ways (Honikel and Hamm, 1994). A small part of the water in meat is the *constitutional water* which comprises about 0.5 g per 100 g protein, i.e. about 0.1 % of the total tissue water that is located within the protein molecules. Protein to water energies for this water are much greater than those existing in normal water binding (Fennema, 1977). A further part of tissue water (5 to 10 % of total water) shows a relatively restricted mobility (Hamm, 1975). According to the classification proposed by Fennema (1977), this fraction of tissue water might be defined as *interfacial water*. This water is bound at the surface of the proteins, probably in multilayers and in small crevices. The interactions result in decreased mobility of the water molecules and represent an intermediate stage between *constitutional water* and the bulk water with a reduced vapour pressure and melting point. A part of this water remains liquid even after freezing below  $-20^{\circ}\text{C}$ .

Nuclear magnetic resonance (NMR) studies and the interpretation of its data raised a number of issues. Numerous authors believe that essentially all of the water in skeletal muscle exists in a physical state different from that of pure water or diluted electrolyte solutions (Blanshard and Derbyshire, 1975; Peschel and Belouschek, 1979). The alternative model, again citing NMR evidence, claims that most of the muscle water behaves like bulk water in a diluted salt solution but that only a small fraction of the water adjacent to the protein molecules has modified molecular structure. This fraction of

tissue water would correspond to the *constitutional* and *interfacial* water mentioned above. Pearson *et al* (1974) agree, giving evidence that water can be held in the myofibrillar complex in two ways according to “bound” or “free”. During the onset of rigor mortis, there is little change in the bound water but the proportion of so-called free water in the extracellular water increases. Lawrie (1998) suggests that interfilament spacing determines the major water-holding capacity of myofibrils, and that spacing is mainly determined by long range electrostatic forces. Most of the water is held in the interfilament between myosin and actin/tropomyosin. This interfilament space is dependent upon pH, sarcomere length, ionic strength, osmotic pressure and whether the muscle is pre-or post-rigor (Offer and Trinick, 1983). This space varying from 320 Å to 570 Å and accounts for a threefold change in the interfilament water content due to binding of water to muscle proteins.

### 1.3 Functional ingredients

#### 1.3.1 Sodium chloride (NaCl)

Sodium chloride is the only salt used extensively in processed and restructured meat products and is the only one legally permitted in all meat products. Currently, there is interest in replacing some of the sodium chloride in meat products with other salt such as potassium or magnesium chloride because consumers have expressed concern about the effect of dietary sodium on hypertension.

As early as 1930, Callow studied the effect of brine salt on small pieces of pork. He found that, with solutions of 25 % salt, meat swelled. This swelling was attributed to a rapid diffusion of salt into the meat with a slow movement of salt soluble proteins outward. Callow (1931) also discovered that the amount of protein extracted is dependent on the salt concentration, with the maximum loss occurring with a 6 to 9 % salt solution. Lawrie (1966) described that with brine solutions (6 to 9 %) there is an initial flow of water-soluble proteins from the meat to the brine coupled with an inward flow of salt. The salt forms a complex with the meat proteins that has a higher osmotic pressure than the brine, thus reversing the flow of water and salt soluble proteins.

Traditionally, these changes in functional properties of meat proteins have been attributed to changes in the pH and /or ionic strength that explain some but not all of these changes in functionality. For example, the extent to which sodium chloride increase functionality is directly related to the increase in ionic strength produced by the salt (Hamm, 1970). Supposedly, chloride ions tend to penetrate into the myofilaments (Figure 2) causing them to swell (Hamm, 1971), and the sodium ions form an ion “cloud” around the filaments (Offer et al, 1989a). This results in local concentration differences that lead to an increased osmotic pressure within the myofibrils, causing the filament lattice to swell. Simultaneously, the enlargement of negative net charge within the myosin filaments loosens the filament at an ionic strength of 0.8 (without added phosphate) or of 0.4 (with added phosphate). However, ionic strength does not completely explain the salt induced changes in meat protein functionality because ionic strength is a measure of how salts change electrostatic interactions that stabilize the muscle protein structure. The electrostatic interactions have a relatively small affect compared to the changes in hydrophobic interactions. Several authors have pointed out that salts only affect these electrostatic interactions when the ionic strength is below 0.1 (von Hippel and Schleitch, 1969; Franks and Eagland, 1975; Melander and Horvath, 1977). At ionic strengths greater than 0.1, the layer of ions surrounding the charged groups on proteins is increased to the point where it shields the charged groups and prevents them from interacting electrostatically with each other. These authors also point out that the changes in protein conformation that occur at ionic strengths greater than 0.1 are due to the changes in hydrophobic interactions, which are major forces stabilising protein structure. According to Schmidt *et al* (1981), the addition of salt produces two effects: the swelling and dissolving of myofibrillar proteins increasing the amount of immobilized water, i.e. increases water retention and, the increase in surface hydrophobicity of muscle proteins leading to fat binding and gel formation on heating.

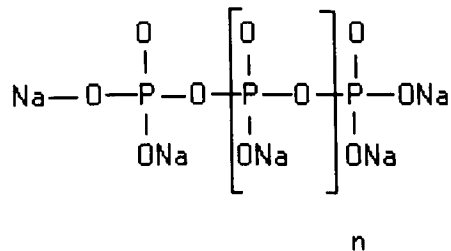
Honikel (1996) states that in comminuted meat, firstly salt facilitates the release of structural proteins from the muscle cells at the surface of the meat during mechanical treatment. Once released, these structural proteins form a tacky layer on the outside of the



meat pieces; and secondly salts interact with the muscle proteins during cooking, causing them to form a strong three-dimensional matrix that entraps free water and binds meat pieces together. Cutting (comminution or mincing) disrupts the cellular and myofibrillar structure. Opening up the myofibrillar structure facilitates the action of salt and water, transforming the meat from a state of limited swelling to that of unlimited swelling as explained by Hamm (1975). Salt ions decrease the attractive forces between adjacent protein molecules, and the added water allows an increase in volume. Meat with its ordered myofibrils has only a limited swelling capacity and, in order to increase it, meat pieces have to be mechanically massaged or tumbled. Honikel (1996) goes further, adding that the addition of salt also causes a conformational change of the myofibrillar proteins which increases the surface hydrophobicity. This increase facilitates on one hand the interaction between protein and fat particles, forming a covering layer around the fat globules, and also allows the hydrophobic regions of the proteins to interact with each other forming upon heating three dimensional structures which support gel formation.

### 1.3.2 Phosphates

Early studies (Bendall, 1954) on the use of polyphosphate prompted work in the 1960s and 1970s to examine the possibility of improving the juiciness and succulence of chicken meat by injecting water and polyphosphates at low levels (Griffiths and Wilkinson, 1978; Grey *et al*, 1996). Although polyphosphate injection in whole muscle is no longer used in the UK poultry industry, polyphosphate is still widely used, often in conjunction with salt, to increase water holding and reduce cooking losses in comminuted products such as chicken nuggets and grillsteaks. Food-grade phosphates used in meat products have a common structure (Figure 7). All are either sodium or potassium salts of different phosphoric acids composed of one or more phosphate units joined together. The condensed or polymeric phosphates contain more than one orthophosphate unit. The most widely used condensed phosphates have the following common names and chain lengths: pyrophosphate, 2; tripolyphosphate, 3; tetrapolyphosphate, 4 to 10; and hexametaphosphate, 10 to 15 (Ellinger, 1972).



**Figure 7** The general structure of straight-chain phosphates (chain length =  $n + 2$ )

Phosphates are used to influence the functional properties of restructured meat products. They are added in solution, either directly to the product during mixing or by needle-injecting the phosphate solution into the meat pieces before mechanical treatment. Phosphates increase the functional properties of restructured products much more than other salts. Therefore, phosphates are used in restructured meat products at much lower concentrations (typically 0.2 to 0.5 %) than salts. Even when used at these low concentrations, phosphates increase the strength of the protein matrix, and hence promote cohesion between the meat pieces more than the highest practical concentration of sodium chloride (Trout and Schmidt, 1984).

The reason why phosphates increase functionality more than other salts is not completely understood. Salts and phosphates increase functionality by a similar mechanism, that is, by increasing extraction of structural myofibrillar proteins and by interacting with these muscle proteins during heating to form a strong heat set matrix (Siegel and Schmidt, 1979a). Part of the reason why phosphates increase functionality can be explained by the increase in pH of the meat. When the pH is increased this increases the extraction of myofibrillar proteins and their subsequent binding capability. Bendall (1954), Yasui *et al*, (1964), and Honikel (1996) have postulated that diphosphate acts as an ATP- analogous substance causing the dissociation of actomyosin in post rigor meat and thus increasing the swelling capacity of the myofibrillar system in the presence of salt. The addition of diphosphate enhances the swelling capacity of meat as it occurs in pre-rigor meat, when ATP is still present.

Electron micrographs of salt and polyphosphate treated raw meats (Lewis, 1981) show that the regions of the sarcomeres most affected are those where actin and myosin would be expected not to be complexed as actomyosin. In particular the H-zones tend to be most dispersed. After heating a pattern of narrow darkly stained bands are produced. These dark bands are thought to originate from the region of overlap between the A and I bands (Figure 4), this region is where actin and myosin are most likely to form actomyosin in meat. Thus the most resistant region of the sarcomere even after treatment with salt and polyphosphate seems to be where actomyosin is formed.

The action of phosphate can also be attributed to an increase in ionic strength in meat products (Schmidt and Trout, 1982; Trout and Schmidt, 1984; Lewis *et al*, 1986). This increase in ionic strength produces a similar action to that of salt. Microscopic observations (Lewis *et al*, 1986) show that, with the addition of salt alone, the features of the muscle are not as clearly defined as those without the addition of salt. This was related to the precipitation of sarcoplasmic proteins within the sarcomeres (Figure 2). After treating the meat with polyphosphate, the myofibrillar proteins within the sarcomeres became more clearly defined, suggesting that the polyphosphate is dispersing the precipitated sarcoplasmic proteins. Dispersion of the precipitated sarcoplasmic protein from the actin and myosin filaments would allow the salt to disperse these proteins more effectively. On heating the dispersed myofibrillar proteins form a gel which retains water within the meat structure. Improved water holding capacity through the formation of myofibrillar gels is supported by many others (Velinov *et al*, 1990; Wang and Smith, 1992; Nielsen *et al*, 1995; Moiseev and Cornforth, 1997; Nowsad *et al*, 2000; Mielnik *et al*, 2002; Tsai-Fuh Tseng *et al*, 2002) and not only in poultry meat, but also in pork (Sheard *et al*, 1999; Fernández-Martín *et al*, 2002), and in beef (Boles and Swan, 2002). The formation of myofibrillar gels also increases the binding capacity of the comminuted meat (Siegel and Schmidt, 1979a; Nielsen *et al*, 1995; Nowsad *et al*, 2000; Mielnik *et al*, 2002).

By understanding the mechanism and the key factors which influence muscle protein functionality, it may be possible to manipulate product formulations and processing

conditions to improve the quality of existing further processed poultry products and to produce new products.

### 1.3.3 Meat emulsions

Meat emulsions are the basis for all comminuted poultry products such as burgers, chicken balls, nuggets, sausage and ready meal components. Gordon and Barbut (1992a) describe a meat emulsion as a complex multiphasic system consisting of solubilized muscle proteins, muscle fibres, fat cells, fat droplets, water, salt and other ingredients. One of the most important functions of a meat emulsion is its ability to hold fat in a stable environment.

Two theories exist which can explain fat stabilization within these matrices:

#### 1.3.3.1 The emulsion theory

A classic emulsion consists of two immiscible liquid phases, one of which is dispersed in the form of a colloidal suspension (Dickinson, 1992). Emulsions are thermodynamically unstable because the free energy of the dispersion is higher than that of the separated liquid phases. Proteins are amphiphilic molecules used in many food systems as emulsion stabilizers. They have the ability to adsorb at interfaces, form continuous protein films surrounding lipid droplets and reduce interfacial tension (Dickinson, 1992).

The emulsion theory has several flaws in that, except for the finely comminuted products, fat droplets are greater than 1  $\mu\text{m}$  and are beyond the colloidal size range of oil droplets found in true emulsions (Dickinson, 1992). Dependent on the type of processing, temperatures may be low enough for the fat to crystallise, separating itself from the traditional idea of a liquid medium in a true emulsion. Indeed, if the product is coarsely comminuted the integrity of the fat cell will be maintained.

Micrographs of raw and cooked emulsion show a proteinaceous membrane surrounding the lipid particles (interfacial protein films) (IPF) (Borchert *et al*, 1976; Gordon and

Barbut, 1990). However, not all fat particles are of uniform size or uniformly surrounded by a protein film. Furthermore, the properties of the interfacial protein films in emulsions are greatly affected by the physiochemical properties of the emulsifying protein, the environmental condition and the method of emulsion formation (Morrissey *et al*, 1987; Dickinson, 1992).

Myofibrillar proteins play an important role in interfacial film formation in meat batters. Galluzzo and Regenstein (1978), using timed emulsion studies, showed that myosin is rapidly taken up at the fat-aqueous interface, followed by actomyosin, troponin, tropomyosin and actin. Photo-micrographs show that myosin forms delicate glutinous emulsions which include small globular structures of uniform size that are little affected upon cooking. Actin forms coarse thin emulsions composed of larger globules of a greater size range, which often undergo coalescence on cooking (Tsai *et al*, 1972; Galluzzo and Regenstein, 1978).

#### 1.3.3.2 Emulsion stability

The droplets of the dispersed phase tend to cluster together spontaneously, forming small or large flocs (flocculation), to coalesce giving larger spherical droplets and to cream (gravitational separation), leading to a layer of the lower density phase on the top of the emulsion (Kinsella, 1984; Leman and Kinsella, 1989). These different processes, which are responsible for the destabilisation and the ultimate oiling off (breaking) of the emulsion system, are interrelated and can act simultaneously giving rise to numerous intermediate physical states, ranging from a perfectly uniform dispersion to completely separated phases. It is also possible to study the kinetics of the stability of an emulsion (Dickinson and Stainsby, 1982) because in a stable dispersed system, rate and extent of change in its structure and properties is sufficiently low in real time (Tolstoguzov, 1992). The rate at which the destabilisation occurs depends on different physical and chemical factors (droplet size and distribution, viscosity of the continuous phase, dispersed phase volume to total volume ratio, specific gravity of the phases, temperature) and these properties are modified by the presence of substances, generally recognised as

emulsifiers, which form an interfacial film between the continuous and dispersed phase (Pearce and Kinsella, 1978; Leman and Kinsella, 1989). This seems to be a circular argument as emulsifiers will affect droplet size and maybe continuous phase viscosity. The key to stability lies in the properties of the surface active components present in the system. Foods contain many surface active ingredients, the most important being proteins and low molecular weight emulsifiers.

Several methods have been proposed to estimate the emulsion stability they have evaluated the chemical, physico chemical and physical changes that in bulk emulsion occur either spontaneously, in the accelerated shelf life tests, under thermal (high or low temperature) or mechanical stresses (Acton and Saffle, 1971; Pearce and Kinsella, 1978; Kato *et al*, 1985; Leman and Kinsella, 1989). The phenomena causing the instability of the food emulsions occur during the different steps of the process and storage, and the resulting changes, in general, give a decrease in the quality of the emulsion.

#### 1.3.3.3 Physical entrapment theory

This theory proposed that the fat phase is entrapped in a three dimensional protein gel matrix after heating (Morrissey *et al*, 1987; Gordon and Barbut, 1992b). When muscle is comminuted in the presence of salt and water, there is an increase in the viscosity of the mix through protein solubilisation, fracturing of muscle structure and swelling of muscle fibre. The viscosity developed in the continuous phase of the raw batter helps to stabilize the dispersed fat by physically retarding coalescence. During thermal processing, myofibrillar proteins in the continuous phase undergo conformational changes leading to the formation of a three dimensional gel matrix which physically entraps water and fat particles (Morrissey *et al*, 1987; Gordon and Barbut, 1992b).

Smith (1988) suggested that both emulsification and physical entrapment may be important in certain processed products: an interfacial protein film may stabilize fat droplets of highly comminuted products whilst in hold tanks or during filling, whereas physical entrapment may be more important in coarse cut sausages prior to cooking and in all further processed poultry products during and after cooking. The relative

contribution of each factor also depends on environmental factors (e.g. pH and ionic strength), physical state of the fat phase (e.g. fat particle size, the melting point of the fat), and the temperature history of the lean phase and processing conditions (e.g. final chopping temperature) (Schut, 1976; Gordon and Barbut, 1992b). Although the relative importance of these two mechanisms of fat stabilization in meat emulsions is not yet clearly understood, current evidence strongly favours the physical entrapment model as the primary mechanism of meat batter stabilization in processed poultry products.

The type and the action of the emulsion will be dependent on the type a product being produced. The UK sausage is a coarse mixture in which 'emulsification' is mostly absent (Evans and Ranken, 1975) whereas a nugget type product would be closer to a true emulsion (Gordon and Barbut, 1990; Koolmees *et al*, 1993; Mielnik *et al*, 2002).

#### *1.3.4 Contribution of muscle protein fractions to meat toughness*

Muscle is a very complex system, the components of which are capable of many and varied interactions during cooking. In spite of this complexity, meat toughness is associated with two protein fractions, the stromal (or connective tissue) and the myofibrillar (Bouton *et al*, 1977).

#### *1.3.5 Stromal proteins*

The contribution to meat toughness of the stromal fraction, of which collagen of the connective tissues is the principal component, is largely dependent upon the severity of heat treatment during cooking, the amount of collagen present, the collagen type, and the incidence of intermolecular crosslinks. Upon heating to 60 to 65 °C, collagen undergoes a characteristic shrinkage as a result of a collapse of the tertiary structure of molecules within the fibre. The temperature at which shrinkage occurs in intramuscular connective

tissue increases with increasing age of the animal and decreases during the postmortem period (Judge and Aberle, 1982). As previously mentioned (Section 1.1.5), in meat from young animals, the contribution of connective tissue to toughness decreases as the cooking temperature is raised above about 50 °C, and is very small by about 70 °C. In the case of very old animals, connective tissue toughness does not decrease until the cooking temperature is raised to about 60 °C, and is relatively large at 70 °C (Bouton *et al*, 1982).

### 1.3.6 Myofibrillar proteins

Most theories propose that with an increase in the overlap of thick and thin filaments the heat-denatured system becomes stronger, and various ways by which this strengthening might occur have been suggested. Voyle (1969) suggested that the effect may be due to an increasing incidence of sarcomeres in which thick filaments of myosin are compressed into the Z-line, thus removing the I-band as a zone of weakness. Goll *et al* (1974) have proposed that the strengthening is due to an increase in the myosin crossbridges attached to actin filaments, and Marsh and Carse (1974) have proposed that it arises from a change in the degree of overlap of actin and myosin filaments.

The belief that the I-band is a zone of weakness has received support from studies of the effect of heat on the ultrastructure of muscle. Locker and Wild (1982) have claimed that the filaments of the A-band can be dissociated by heat. The response of the I-band to heat can vary considerably: for instance, in turkey muscle heated to 82 °C the I-band remains intact in the *M. semitendinosus*, a red muscle, but is removed in the *M. pectoralis major*, a white muscle (Dahlin *et al*, 1976).

An alternative theory to those based on thick and thin filaments to account for contraction state toughness has been discussed in a series of papers by Locker *et al* (1977); Locker and Wild (1982) and Locker and Wild (1984). Locker and Wild (1982) believed that the thick and the thin filaments are dissociated when meat is cooked and therefore the concept of the A-band in cooked meat as a fused structure of coagulated A-filaments has to be abandoned. They propose that the myofibrillar component of meat is related to a third set of filaments in the sarcomere, which they called “gap filaments” (Section 1.1.4).



If gap filaments are composed principally of titin, it is difficult to reconcile the apparent heat lability of titin, which is unaffected by the contraction state of muscle, with the large contraction state has on the toughness of the cooked muscle (King, 1984). No disappearance of titin from chicken muscle and no significant differences in the content and electrophoretic pattern of titin isolated from chicken muscle during conditioning were reported by Locker and Wild (1984); Fritz *et al* (1993) and Suzuki *et al* (2001).

An increase in the ultimate pH of muscle results in a decrease in the myofibrillar component of toughness to the extent that this component is negligible in muscle with pH values near 7.0 (Bouton *et al*, 1982).

## **1.4 Function of meat proteins in restructured meats**

### *1.4.1 Sarcoplasmic proteins*

The inferior binding properties of the sarcoplasmic proteins have been demonstrated in several studies. Acton and McCaskill (1972) concluded that, with respect to increasing binding strength, the quantity of water-soluble proteins available appears less important than the quantity of salt-soluble proteins. Macfarlane *et al* (1977) and Ford *et al* (1978) investigated the effectiveness of sarcoplasmic proteins as binding agents by using them to form a junction between two pieces of meat. They found that the binding achieved by sarcoplasmic proteins is slight.

### *1.4.2 Myofibrillar proteins*

The binding of the principal protein of the myofibril, myosin, has long been recognised as important. Most studies have focused on the ability of the proteins to bind meat particles into a cohesive mass when cooked. The abundance of myosin in the myofibrillar fraction, its demonstrated ability to bind meat pieces, and its potential for achieving high binding strength account for the strong interest in the characteristics of the heat denaturation of myosin. After myosin, actin is the next most abundant of the myofibrillar proteins. Samejima *et al* (1969;1980) showed that actin can strongly associate with myosin to form

actomyosin. Actin itself demonstrates poor binding ability. Its associations with myosin can influence and are important in relation to the heat denaturation of myosin.

### 1.4.3 Gelation of myosin

When poultry myosin is heated in solution in 6.0 M KCl or NaCl at pH 6 it forms a gel, the rigidity of which increases with the temperature of heating over the range 35 to 60 °C (Ishioroshi *et al*, 1979). The optimum temperature and pH for gelation are 60 to 70 °C and pH 6. The occurrence of two transition temperatures (43 and 55 °C) in the rigidity versus temperature plots indicates that the gelling reaction involves at least two conformational changes of the molecule. However, Samejima *et al* (1981) employed dithiothreitol, an inhibitor of disulphide bond formation, to remove the transition in myosin at 43 °C. This indicated that this transition originates from the aggregation of the head region of the myosin molecule because disulphide links appear to be formed only between the head and not between the rod regions of myosin molecules on heating (Samejima *et al*, 1984).

#### 1.4.3.1 Effect of muscle type on gelation

Poultry comminutes are commonly mixtures of breast and thigh muscle (Mandava and Hoogenkamp, 1999) products made with more thigh than breast tend to be cheaper. Using differential scanning calorimetry, Liu *et al* (1996) reported that *M. pectoralis* myosin undergoes a thermal transition at 48.1 °C, whereas the first thermal transition of the *M. iliotibialis* and *M.gastrocnemius* myosin were at 49.5 and 49.3 °C, respectively, in 0.6 M NaCl, 0.05 M sodium phosphate buffer, pH 6.0. Liu *et al* (1996) using isothermal heating experiments suggested that only *M. pectoralis* aggregated on heating at 45 °C. *M. iliotibialis* and *M.gastrocnemius* did not start to aggregate until heated isothermally at 50 °C. Asghar *et al* (1984) reported that myosin extracted from *M. pectoralis profundus* (in 0.6 M NaCl, pH 6.0) formed more rigid gels than myosin from *M.gastrocnemius* at 65 °C at pH 5.1 to 6.0.

#### 1.4.3.2 Effect of actin, actomyosin and tropomyosin on myosin gelation

Inclusion of actin at the appropriate levels makes a significant contribution to the gel forming characteristics of myosin heated in salt solution (Samejima *et al*, 1969; Nakayama and Sato, 1971a; Samejima *et al*, 1980; Yasui *et al*, 1980). When actin is mixed with myosin at pH 6 and in 0.6 M KCl, a synergistic increase in gel strength occurs. When heated under these conditions of pH and salt concentration F-actin does not show signs of gelation, but instead precipitates. The synergistic effects of the addition of actin on myosin gelation are most pronounced in solutions at pH 5.5 to 6.0 and with 0.5 to 0.8 m salt (KCl). Yasui *et al* (1980) found that mixtures of myosin with actomyosin also exhibit a synergistic increase in gel rigidity, the maximum effect occurring at a myosin: actomyosin weight ratio of about 4. Binding quality is supposed to be improved in the presence of the regulatory protein tropomyosin (Nakayama and Sato, 1971b). However, Samejima *et al* (1982) from studies of the heat induced gelation of natural and desensitised actomyosin and Samejima *et al* (1981) from studies of reconstituted actomyosins in the presence and the absence of tropomyosin concluded that tropomyosin does not affect the gel structure of the actomyosin system.

### 1.5 pH drop

The function of muscles is to contract. The fuel for contraction and maintaining the functional integrity of muscle comes in the form of Adenosine Triphosphate (ATP). The actual level of ATP at any one time is very low. However, ATP is constantly maintained by the Lohmanns reaction:-



**Equation 1** Lohmanns reaction (Warris, 1996) CP = Creatine Phosphate, ADP = Adenosine Diphosphate, CPK = Creatine Phosphokinase, C = Creatine, ATP = Adenosine Triphosphate

In comparison with respiration, post-mortem glycolysis is an inefficient means of providing energy. The yield of ATP during post-mortem glycolysis is only 2 to 3 mol of ATP per mol of glucose, compared with 36 or 37 mol during aerobic respiration. The drop of the pH in muscle post-mortem is brought about by the breakdown of one moiety of glycogen to two molecules of lactic acid. As lactic acid accumulates, muscle pH declines from  $> 7.1$  to a metabolic ultimate pH 5.4 (de Fremery and Lineweaver, 1962; Stewart *et al*, 1984; McGinnis *et al*, 1989), below which glycolysis is inhibited. Consequently, the rate and extent of the pH decline and the quantity of lactic acid accumulated depend on the amount of glycogen present in the tissue at the time of death. The onset of rigor is not triggered at a specific pH but occurs irrespective of pH when  $> 60\%$  of initial ATP is utilised (Khan, 1976). In the subsequent rapid phase of rigor, there is abrupt sarcomere shortening and decrease in extensibility. The pH at the onset of the rapid phase is linearly related to ultimate pH, which is determined by the initial glycogen reserves of the muscle as the time of slaughter ( $\text{pH onset} = 0.76 (\text{ultimate pH} + 2.2)$ ) (Bate-Smith and Bendall, 1949). The rate of pH change at any pH (between 7.0 and 5.8) is consistent from animal to animal at a uniform temperature, with minimal rate at pH 6.7. Minimal change in muscle tension also occurs within the pH range 6.7 to 6.4, and the sarcoplasmic reticulum continues to sequester calcium. When a muscle is in full rigor, the shortest sarcomere lengths are attained (1.4 to 2.0  $\mu\text{m}$ ) at 2 to 8 h post-mortem and the muscle becomes extensible (Khan, 1976; Dunn *et al*, 1993). Full rigor for breast fillets (complete inextensibility of muscles) occurs at 2 to 4 h post-mortem (pH 5.6 to 5.4) and for leg muscles by 2 h (pH 6.0 to 5.9) (Kijowski *et al*, 1982). The longer onset and pH decline seen in breast is due to the increased initial ATP reserves. Initial ATP levels are also higher in chicken fillets (4.8  $\text{mg g}^{-1}$ ) than beef muscles (3.1  $\text{mg g}^{-1}$ ). Rigor mortis not only develops, but resolves earlier post-mortem in leg muscles than in breast muscles (Kijowski *et al*, 1982).

Variations in the rate and extent of rigor mortis affect the physical qualities of poultry meat. A high final pH produces dark, firm and dry poultry meat with a short shelf life while low final pH produces meat with poor water holding and a low colour intensity

(Allen *et al*, 1996; Fletcher, 1999). Accelerated rigor mortis enhances protein and pigment denaturation leading to pale, soft and exudative meat (PSE), well known in pigs.

## 1.6 Muscle fibre type

### 1.6.1 Nomenclature

George and Berger (1966) in their monograph on comparative avian myology showed that the distribution of aerobic and anaerobic enzymes between muscle fibres could be correlated with muscle structure and function. During the past 40 years there have been widespread studies on muscle fibre types both on the cellular and molecular level (Cassens and Cooper, 1971; Skorjanc *et al*, 1997). Muscle fibres can be classified according to their metabolic, contractile and colour properties. However, a confusing number of taxonomic systems of fibre typing have been described. Commonly bipartite systems such as red-white and tripartite systems such as red-intermediate-white have been proposed, leading to large numbers of categories.

Gauthier (1969) used a method of fibre typing based on histochemical reactions of aerobic oxidative capacity, using Succinate Dehydrogenase (SDH). Three major fibre types were distinguished: red, intermediate and white reflecting differences in mitochondrial content. Brooke and Kaiser (1970) developed a histochemical classification of muscle fibres based on the sensitivity of Adenosine Triphosphate (ATPase) activity after exposure to either high (>10) or low (<4) pH. The following types can be delineated on the basis of myofibrillar ATPase (mATPase) activity after pre-incubation: I, IIA, and IIB (IIC). Combining the oxidative SDH staining with the ATPase activity, Ashmore and Doerr (1971a) proposed three fibre types:  $\beta$ R, ATPase acido-stable, and oxidative;  $\alpha$ R, acido-labile and oxidative;  $\alpha$ W, ATPase acido-labile and glycolytic.

Combination of the histochemical stains for the oxidative enzyme nicotinamide adenine dinucleotide dehydrogenase (NADHase), terazolium reductase, and ATPase, also resulted in three major fibre types: slow-twitch oxidative (SO), fast-twitch oxidative glycolytic

(FOG) and fast-twitch glycolytic (FG) (Peter *et al*, 1972). Unfortunately the classification systems based on stains for enzymes involved in oxidative metabolism (Peter *et al*, 1972) and mATPase activity (Brooke and Kaiser, 1970) appear to be incompatible. The SO fibres correspond to type I, but FG and FOG fibres do not fully match fibre types IIA, IIB or IIC (Pette and Staron, 1990). Essén-Gustavsson and Lindholm (1984) classified various pig muscle according to ATPase (I, IIA and IIB) and NADH-tetrazolium reductase finding that 15-20 % of type IIB fibres in *M. longissimus* stained medium for NADH-tetrazolium reductase. Fernandez *et al* (1995) concurs and, using both ATPase and SDH methods, demonstrated that 7 % of type IIB fibres in *M. longissimus lumborum* stained positively for SDH and would, using Ashmore and Doerr (1971b) classification, be  $\alpha$ R fibre types. For the purpose of this thesis the nomenclature of Pette and Staron (1990) Type IIB (white/fast); Type I (red/slow); Type IIA (white/intermediate) will be used.

Thus muscle fibre types differ phenotypically in that they not only express different subsets of myofibrillar isoform genes with different specific ATPase activities but also different types and levels of metabolic enzymes. The fact that there are several myosin isoform genes could mean that muscle fibres possess the ability to alter their contractile properties during development and in response to levels of activity (Moore and Goldspink, 1985; Goldspink and Scutt, 1992). This involves both quantitative and qualitative changes in gene expression, which results in alteration in the cross-sectional area of a given type of fibre.

### 1.6.2 Muscle morphology and variation

An important point when analysing muscle fibre type composition of a muscle and its relation to meat quality are the structural differences associated with different fibre types and the variation in fibre types within muscles. Most research shows an inverse relationship between fibre diameter and the oxidative capacity of muscle fibres. Type I are the smallest, type IIB fibres have the largest diameter, and IIA have an intermediate size (Cassens and Cooper, 1971; Rosser *et al*, 1992). Moreover, type I and IIA fibres

have a greater lipid and myoglobin content and more capillaries per fibre than type IIB fibres (Essén-Gustavsson *et al*, 1992). The combination of fibre type, size and capillarisation is important in relation to the post-mortem muscle metabolism and meat quality (Cassens *et al*, 1975; Henckel, 1995). Capillary density is rarely mentioned in research papers except in a passing interest to SDH and oxidative capacity (Swatland, 1983). Ante-mortem muscle depends to a large extent on oxygen supply and the potential to remove waste products such as lactate, thus affecting the rate and extent of rigor mortis. Assessment of capillary density gives estimates of the upper capacity for blood flow (Saltin and Gollnick, 1985).

Variation of fibre type distribution within muscle is also of importance when studying fibre type composition in relation to meat quality. Muscles involved in posture are more oxidative than those involved in movements (Totland and Kryvi, 1991; Henckel, 1995). In pigs, it was demonstrated that the deepest muscles of the limbs generally have the highest percentage of slow oxidative type I fibres, while the superficial muscles have the highest percentage of glycolytic (Type IIB) fibres (Armstrong *et al*, 1987). Brandstetter *et al* (1997) found similar results for beef *M. semitendinosus* by analysing metabolic enzymes and MHC isoforms. Moreover, deep IIB fibres are more oxidative and have smaller diameters than superficially situated type IIB fibres (Rosser *et al*, 1992).

### 1.6.3 Effects of selection

Muscle fibre type composition and structure are genetically defined and can be influenced by environmental factors, such as physical activity and housing systems (Stecchini *et al*, 1990; Petersen *et al*, 1997a), nutrition (Karlsoon *et al*, 1994), climatic circumstances (Herpin and Lefaucheur, 1992), and administration of specific growth promoting agents (Rehfeldt and Ender, 1993; Solomon *et al*, 1994; Petersen *et al*, 1997a; Petersen *et al*, 1997b). The main difference described is that between the layer-type chicken and the broiler-type chicken (Aberle and Stewart, 1983). Broiler birds yield a higher percentage of meat and lower percentage of bone compared to layer birds (Dawson and Walters, 1958). Smith (1963) reported that broiler type birds had larger

diameter muscle fibres than layer birds at 10 weeks of age. Mizuno and Hikami (1971) also found greater numbers of myofibres in broiler than layer birds. Smith (1963) presented indirect evidence that the broiler line had more muscle fibres than the layer birds, but concluded that fibre size was more important than fibre number in determining muscle size in these lines. Undoubtedly, more rapid radial fibre growth is an important contributor to greater muscle growth rate and size, although there is a physiological limit to muscle fibre diameter, and this might mean that broiler-type birds would stop growing at an earlier age. However, broilers may have a different maximum limit to fibre diameter at maturity.

#### 1.6.4 Growth

It is well known that in the chicken, characteristics of the growth of each muscle are unique (Iwamoto and Takahara, 1971; Iwamoto *et al*, 1975; Ono *et al*, 1982). Postnatal growth of skeletal muscle is accompanied by growth of individual muscle fibres, because the number of muscle fibres does not increase after hatching (Smith, 1963; Mizuno and Hikami, 1971). However, there is evidence, in pigs, that increases in apparent fibre numbers have been found postnatally (Swatland, 1975a). In cattle, Bendall and Voyle (1967) found decreases in apparent fibre numbers. Joubert's (1956) data can be calculated to show a corresponding decrease in the apparent fibre number in sheep (Swatland, 1974). Growth of muscle fibres is considered to be controlled by two factors: 1) enlargement by an increase in diameter, mainly due to the accumulation of myofibrils; and 2) elongation due to addition of newly formed sarcomere to the ends (Williams and Goldspink, 1978). In pigs, radial fibre growth is accompanied by an increase in the number of myofibrils seen in cross section (approximately 11 myofibrils/kg live weight gain) with no increase in their mean size (Swatland, 1976).

Proliferation of myofibrils may also occur by longitudinal growth due to the lateral apposition of new myofilaments (Goldspink *et al*, 1970). Longitudinal growth of muscle fibres occurs by the formation of new sarcomeres at the end of each fibre (Muir, 1961). The contribution of radial and longitudinal growth to overall growth is modified by fibre



arrangement (Helmi and Cracraft, 1977). In the *M. pennate supracoracoideus* of ducks, radial fibre growth continues to contribute to overall muscle length and weight even after longitudinal fibre growth has ceased (Swatland, 1980a).

#### 1.6.5 Fibre type changes with animal age

Ashmore and Doerr (1971a) have shown that IIA fibres transform to larger diameter IIB fibres during the development of the chick, and it has been suggested that selection for muscle size in animals may result in an accumulation of factors that promote greater conversion of IIA to IIB fibres during development (Ashmore *et al*, 1972). Suzuki (1978) agrees, stating that the *M. pectoralis thoracicus* muscle of chickens without the ability for long term flight has only very few type I fibres and is in contrast to that of the pigeon, possessing ability for cruising flight. The pigeon *M. pectoralis thoracicus* muscle contains many type I fibres. These fibres are thought to play an important role in long-term locomotion rather than the IIB fibres. Type IIB fibres are believed to be adapted for only short-term intense activity. Muscle fibres corresponding to type IIA fibres have not been found in the pigeon *M. pectoralis thoracicus*. Type IIA fibres were found in the limited, deepest region of the chicken *M. pectoralis thoracicus*. This finding showed that the difference in fibre type composition in the *M. pectoralis thoracicus* exists between chicken and pigeons. The difference in fibre type composition seems to be caused by a functional differentiation in muscle. The mammalian *M. soleus* that are primarily involved in the maintenance of posture have numerous fibres corresponding to type II fibres. Therefore, the flexor *M. cruris medialis* and *M. biventer cervicis* muscles possessing IIA fibres are presumed to be involved in the maintenance of posture in addition to locomotion, whereas the *M. iliotibialis lateralis* muscle composed of type I and IIB fibres appears to function largely during locomotion.

Type II myofibres in the chicken have a faster contraction speed and more capability for anaerobic metabolism than type I myofibres, (Ashmore and Doerr, 1971b). Ashmore *et al*, (1972) suggested that domestication of animals and selection for muscularity

promotes concentration of genetic factors that favour conversion of type II red to type II white myofibres.

At the individual myofibre level, Pette and Staron (1990) suggested a continuum between two extreme types, i.e. types I and IIB. This was further emphasised by the fact that different isoforms of myosin, the protein defining contraction speed of fibres, might coexist within the same fibre or within an individual myofibril (Gauthier, 1990) and even within an individual thick filament (Taylor and Bandman, 1989; Morrison *et al*, 1998). Swatland (2002) concurs stating that quantitatively, the range from aerobic to anaerobic metabolism is usually a continuous variable and is seldom broken into discontinuous steps.

Thus, to some researchers, the histochemical categorization of muscle fibres by any method is a useful but artificial subdivision of a continuously variable range. Concluding that muscle fibres undergo a continual alteration throughout life as an adaptation to changing functional demands and that “fibre type” merely reflects the constitution of a fibre at any particular time.

## **1.7 Aims and outline of the thesis**

The linkage between animal production parameters and meat processing functionality is poorly understood particularly for poultry products. The aims of this project were to describe the relationships between animal production parameters and meat processing functionality, plus the underpinning science of protein functionality under different processing situations.

The experimental work of this project is preceded by a chapter on the materials and methods used throughout (Chapter 2), which includes a pilot study to establish routines for the histochemical demonstrations of muscle fibre type and protein functionality models. The experimental work is divided into 4 chapters. Chapter 3, 4, and 5 describe experiments that examine the effects of sex, together with genotype, age, and diet, on pre-incubation pH (72 h) and three a whole muscle model (WMM), a comminuted muscle

model (CMM), and an oil emulsion model (OEM) different meat functionality models. A long-term experiment, examining the combined affects of sex, genotype, age and diet, including analysis of muscle fibre type, electrophoresis of meat proteins, and microstructure of processed meat from the different functionality models is given in Chapter 6. All experiments will be discussed in relation to one another in Chapter 7

## Chapter 2

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### General Materials and Methods

Many methods used in this thesis have been employed repeatedly. The following information details materials and techniques used regularly, in order to avoid repetitious explanations.

#### 2.1 Subjects

Birds were Ross 308 (broiler-type) and Hubbard JA57 (layer-type). Apart from the pilot study the birds were removed from a carcass evaluation unit. Individual birds were identified using wing tags, or different coloured leg rings. Three different leg ring sizes (6.4, 8, and 14 mm) were used to accommodate leg growth.

#### 2.2 Husbandry

##### 2.2.1 Housing

General commercial practices were carried out with regards to heating, lighting and feeding and these are detailed in Appendix A. Birds were either taken from commercial stock (pilot study 2.3) or removed from study groups outlined in each chapter. Pen floors were covered in litter (woodshavings). There was one bell drinker and feeder provided per pen, containing *ad libitum* supplies of water and food. Their diet consisted of a commercial broiler breeder diet unless otherwise specified (Experiment 3 and 4, Appendix A).

#### 2.3 Pilot study

An initial pilot study was conducted to establish routines for the protein functionality models, electrophoresis, microscopy and histochemical demonstrations of muscle fibre type. It was also necessary to develop existing histochemical techniques to allow image analysis software to collect quantitative data from muscle sections. A total of 15 male Ross 308 broilers were stunned and slaughtered under normal commercial conditions at

an on-site carcass evaluation unit. After exsanguination had finished (120 sec), the birds were removed from the line to a laboratory for sampling.

### 2.3.1 *Histological muscle fibre typing*

The *M. pectoralis major* (breast) was chosen because it is predominately composed of type IIB muscle fibres (Ashmore and Doerr, 1971a; Ouail, 1999; Hoving-Bolink *et al*, 2000). The breast muscle is also of economic importance as the most profitable muscle on the carcass (Mandava and Hoogenkamp, 1999). The *M. iliotibialis* (thigh) was chosen as it composed of a mixture of muscle types (Ashmore and Doerr, 1971a; Ouail, 1999; Hoving-Bolink *et al*, 2000).

The *M. pectoralis* and the *M. iliotibialis* were exposed by first splitting then pulling back the skin. Blocks of muscle were excised using a surgical scalpel. The *M. pectoralis* blocks were removed from the external face of the breast in a location near the keel of the sternum, midway between the cranial and caudal edges of the muscle. *M. iliotibialis* blocks were removed from the external face of the thigh at the postdorsal region near the trochanter of the femur. The muscle was then fixed in accordance with Ashmore and Doerr (1971a) and stained in accordance with Guth and Samaha (1970) and Neuromuscular (2002).

### 2.3.2 *Sample freezing*

Following the Ashmore and Doerr (1971a) method, the muscle samples were dipped in talcum powder (BDH 14807-96-6) then immersed into liquid nitrogen until the sample stopped bubbling. The frozen muscle blocks were then removed using forceps, sealed in bags and stored at  $-30^{\circ}\text{C}$  until processing.

### 2.3.3 Cutting

The frozen muscle blocks were mounted on a cryostat chuck (Cryo-cut E, Austria) with cryogel (Tryco-m-bed, AMC, London) such that muscle fibres were perpendicular to the cutting blade, and allowed to equilibrate to  $-20^{\circ}\text{C}$ . Serial sections  $12\ \mu\text{m}$  thick were cut and the frozen sections were allowed to dry on microscope slides taken from room temperature. Whilst other sections were being processed, the cut sections were placed in microscope slide rack and held at  $-20^{\circ}\text{C}$ . Six sections were cut from each bird, one for acid ATPase activity, one for alkali ATPase activity, one for SDH activity and one control for each method.

### 2.3.4 Staining

The most widely used procedures for histochemical classification (Section 1.6.1) of skeletal muscle fibres into distinct types involves staining one section for determination of functional activity (e.g., myofibrillar adenosine triphosphate (M-ATPase)) activity after either an acid or alkaline pre-incubation), and another for the detection of metabolic capacity, succinic dehydrogenase (SDH).

Slides were placed into a stainless steel cradle that positioned them vertically on their longest axis. The sectioned muscle blocks were then subjected to one of the following histological techniques. One slide each was used for acid and alkali stable ATPase, as this allowed a “positive and negative” stain for each fibre type. An explanation of each solution is given in Sections 2.3.4.3 and 2.3.4.5 for ATP and SDH respectively. All slides were incubated in coplin jars at room temperature unless otherwise specified.

#### 2.3.4.1 Alkali stable Adenosine Triphosphate (ATPase)

1. Immerse sections in solution 1 for 5 min.
2. Rinse in solution 2 for 1 min. Blot dry using Kimwipes® lite.
3. Pre-incubate in solution 3 for 15 min.
4. Rinse slides in solution 2 ( 2 changes, 1 min each) and drain excess solution.

5. Incubate in solution 4 at 37 °C for 60 min.
6. Wash in three 30 sec changes of solution 5 and drain excess solution.
7. Immerse in solution 6 for 3 min.
8. Wash in four 30 sec changes of solution 7 and drain excess solution.
9. Immerse in solution 8 for 3 min.
10. Wash in running tap water for 5 min and dry off excess water.
11. Mount with DPX mounting medium (R1340, Agar Scientific, England), (coverglass, 22 x 22 mm, thickness No1, DBH, England) and allow to dry for 12 h.

(Guth and Samaha, 1970)

#### 2.3.4.2 Acid stable ATPase

1. Prepare frozen sections as per step 1 to 3 above.
2. Pre incubate the unfixed sections in solution 9 for 30 min and drain excess solution.
3. Complete the preparation as per steps 7 to 13 above.

#### 2.3.4.3 Acid and alkali stable ATPase solutions

1. Fixative (5 % formalin buffered at pH 7.6).
2. Rinse solution, mM CaCl in 100 mM tris (hydroxymethyl) aminomethane (Tris).
3. Alkaline pre-incubation (18 mM CaCl in 100 mM buffer, pH 10.4).
4. Incubation solution (2.7 mM ATP, 50 mM KCl, 18 mM CaCl in 100mM buffer, pH 9.4).
5. Wash solution (1% CaCl, weight for volume, w/v).
6. Cobalt chloride solution (2 % w/v).
7. Alkaline washing solution (100mM buffer, pH 9.4).
8. Ammonium sulfide solution (1% w/v).
9. Acid pre-incubation solutions (50 mM potassium acetate, 18 mM CaCl pH 4.35).

#### 2.3.4.4 Succinic Dehydrogenase (SDH)

1. Immerse slide into solution 1 and incubate at 37 °C in a water bath, (Gallenkamp App No 792881, fitted with a Thermo- stirrer 100, EEC) for 1 h.
2. Remove slide from solution 1 and rinse with distilled water for 5 min.
3. Immerse slide in a mixture of 30 % acetone with deionised water for 5 min. Remove and immerse slide in mixture of 60 % acetone with deionised water for 5 min. Remove and immerse slide in a mixture of 90 % acetone with deionised water for 5 min. A faint purple cloud should be seen over the section.

(Neuromuscular, 2002).

#### 2.3.4.5 SDH solutions

1. 0.2 M sodium monobasic phosphate, ( $\text{NaH}_2\text{PO}_4$ ) anhydrous, (27.8 g/litre deionised  $\text{H}_2\text{O}$ ), 13 ml sodium dibasic phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ ) heptahydrate (53.65 g/litre deionised water), 87 ml 0.2 M succinic acid disodium salt, ( $\text{C}_4\text{H}_4\text{O}_4\text{Na}_2 \cdot 6\text{H}_2\text{O}$ ) (5.4 g/litre water), FW 270.1, 270 mg and nitro blue tetrazolium ( $\text{C}_{40}\text{H}_{30}\text{C}_{12}\text{N}_{10}\text{O}_6$ ), 10 mg.

The ATP slides were used for image analysis and the SDH slides were used as controls to contrast against the ATP slides. Using these above fibre typing techniques highlighted a number of problems, described below.

#### 2.3.5 *Muscle volume*

When the muscle fibres were removed from a muscle prior to the onset of rigor mortis, they contracted in a manner that was difficult to control. Even if the sample was restrained at a set length, the muscle fibre length was not maintained. Some of the fibres contracted and some stretched as described by Swatland (2002). The contracted fibres increased their apparent fibre area and the stretched fibre decreased their apparent fibre area.



### 2.3.6 *Difficulty of measuring fibre diameter*

The cross sectional areas of muscle fibres are increased if the fibres are cut obliquely rather than perpendicular. Oblique sections may cause poor alignment of the tissue on the microtome chuck but, even in correctly aligned tissue, they may occur if some fibres of the sample are contracted more than others. Fibres with a lesser degree of contraction may be thrown into sinuous folds that give rise to oblique sections at intervals along the fibre length. Muscle fibres are polygonal, the minimum diameter may underestimate true mean diameters. Also, ice crystal artefact falsely increases the fibre diameter by splitting open the fibres and stretching the surrounding connective tissue.

### 2.3.7 *Image analysis*

The study of muscle fibre histochemistry by subjective (human eye) assessment of reactions can be rather confusing. There are several different systems of categorising histochemical fibre types (Section 1.6.1) and borderline cases have to be assigned arbitrarily to a particular fibre type. Also, the apparent number of fibres appearing in a cross-section may be considerably less than the total number in the whole muscle (real fibre number). Whole fasciculi (Section 1.1.1) may be missed from the plane of sectioning (Swatland, 1975b) and in sectioned fasciculi, there may be intrafascicularly terminating muscle fibres which do not reach the plane of sectioning. Intrafasciculating terminating muscle fibres are muscle fibres which are attached to a tendon at one end, with the other end tapering to a small diameter and anchored in the endomysium around another muscle fibre. Thus, the end diameter of these fibres belies the true muscle fibre diameter.

Therefore, due to the problems explained in Sections 2.3.5, 2.3.6 and 2.3.7 an alternative to these traditional techniques was attempted to allow for an objective and simple means of measuring fibre proportions and types within muscle.

## 2.4 Alternative method (muscle block staining method)

### 2.4.1 Muscle sampling

Immediately after death, the birds skin was pulled back to reveal the muscle. Using a surgical scalpel, blocks of muscle tissue 5 mm<sup>3</sup> in size were excised from the left *M. pectoralis major* and the *M. iliotibialis* of each bird. The *M. pectoralis* blocks were removed from the external face in a location near the keel of the sternum, midway between the cranial and caudal edges of the muscle. *M. iliotibialis* blocks were removed from the external face of the thigh at the postdorsal region near the trochanter of the femur. Each block was cut so that its long axis ran parallel to the muscle fibre direction. This was to reduce the likelihood of oblique sections.

The remainder of the muscle was removed from the bone and sealed in bags (Ziplok®, USA) to prevent desiccation and refrigerated at 1 °C (± 0.5 ) for 72 h.

### 2.4.2 Sample freezing

Using a technique adapted from Hoving-Bolink *et al* (2000), an aluminum dish (5cm wide x 1cm deep) filled with 2-methylbutane (CH<sub>3</sub>)<sub>2</sub>CH CH<sub>2</sub> CH<sub>3</sub>, was floated on the surface of liquid nitrogen. Muscle blocks were dipped into the 2-methylbutane when it became syrupy and immersed for ten seconds. Because the 2-methylbutane is in a semi solid state, it draws the heat from the muscle sample quicker than liquid nitrogen thus reducing the formation of ice crystal artifact. The frozen muscle blocks were then removed using forceps and placed in Beem® cups which were in turn sealed in ziplok® bags to prevent desiccation and stored at -30 °C until processing.

### 2.4.3 Block staining

1. Remove the muscle sample from the Beem® cup and immerse into solution 1 and incubate at 37 °C for 1 h.
2. Remove the muscle block from solution 1 and rinse with distilled water for 5 min.
3. Immerse the muscle block in a mixture of 30 % acetone with deionised water for 5 min. Remove and immerse muscle block in mixture of 60 % acetone with deionised water for 5 min. Remove and immerse muscle block in a mixture of 90 % acetone with deionised water for 5 min.
4. Immerse the muscle block in solution 2 for 30 min.
5. Replace solution 2 with 100 % alcohol and leave for 12 h.
6. Replace the 100 % alcohol with solution 3 and leave for 1 h. Repeat this step after 1 h and leave for 12 h.
7. Replace solution 3 with solution 4 and leave for 1 h. Repeat this step twice and leave for 12 h.
8. Remove the muscle block and place in a Beem® cup, taking care to align fibre direction parallel to cup side wall.
9. Fill Beem® cup with solution 4.
10. Incubate (Gallenhamp, Economy Incubator, size 2, EEC) at 60 °C for 3 h.

### 2.4.4 Solutions

1. 0.2 M sodium monobasic phosphate, ( $\text{NaH}_2\text{PO}_4$ ) anhydrous, (27.8 g/litre deionised water), 13 ml sodium dibasic phosphate ( $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ ) heptahydrate (53.65 g/litre deionised water), 87 ml, 0.2 M, succinic acid disodium salt, ( $\text{C}_4\text{H}_4\text{O}_4\text{Na}_2 \cdot 6\text{H}_2\text{O}$ ) (5.4g/litre  $\text{H}_2\text{O}$ ), FW 270.1, 270 mg and nitro blue tetrazolium ( $\text{C}_{40}\text{H}_{30}\text{C}_{12}\text{N}_{10}\text{O}_6$ ), 10 mg.
2. 50 % alcohol, 50 % acetone.
3. 50 % Alcohol, 50 % London resin white (hard) (100 g resin, 1.98 g catalyst).
4. 100% London resin white (hard) (100 g resin, 1.98 g catalyst).

The fixed muscle block was removed from the Beem® cups using a straightedge razor. Excess resin was removed from the leading edge of the muscle so to expose the fixed muscle tissue underneath. This would allow the optical sectioning of muscle samples Z axis.

Although the block staining technique was successful for the demonstration of SDH (metabolic activity), it was not successful for the demonstration of either acid or alkali stabile ATP (glycolytic capacity). Oxidative capacity alone is not sufficient as a histochemical demonstration of muscle fibre types. Thus, traditional techniques had to be employed for the cutting and the histochemical staining of all muscle sections.

#### 2.4.5 Image analysis

Images per muscle section were captured using a MRC 1000 Confocal Laser Scanning Microscope (CLSM) (Bio Rad, England). The following settings were used:

Objective lens  $\infty/0.17/d$ ; NPLAN 40x/0.65, pH2; Epi detector 1; emission filter open; iris 3.7 mm; gain (v) 910; blacklevel 0; PMT 1 100 %; PMT 2 0 %; transmission 54 %; enhance 3; transdetector gain 0; blacklevel 0. The area of analysed muscle was 390 x 260  $\mu\text{m}$  (pixel size 0.508  $\mu\text{m}$ ). Images were saved as Tagged Image File Format's (TIFF) and the images per section were analysed by computer image analysis (Scion Image J, USA) using a semi-automated procedure, as follows: Each image was calibrated against the Bio Rad software for length and densitometry. Image sections were scanned for total fibre area ( $\mu\text{m}^2$ ) and pixel density of each fibre. The automatic threshold tool did not highlight all muscle fibre boundaries due to the natural variation within individual muscle fibres and between muscle fibre samples. Thus, an alternative threshold was created using the macro;

Macro 'Std Dev threshold [T]';

VAR

Count, Threshold:integer;

StdDev, TheMean, MultFactor:real

```

BEGIN
ResetGrayMap;
MultFactor :=GetNumber('StdDevs past the mean to threshold',1.0)
ResetCounter;
Measure;
StdDev :=rStdDev[rCount];
TheMean:=rMean [rCount];
Threshold := TheMean+ round (Multfactor*StdDev);
SetThreshold (Threshold);
Showmessage ('Threshold level= ',Threshold,'\Using ',MultFactor:4:2, 'Standard
of
deviations','\from the mean');
END;

```

The measurement excluded fibres touching the image boundary.

#### 2.4.6 Densitometry

The density of each fibre was a calculation of the mean of 1000 muscle fibre scans. The CLSM was used to capture images of the muscle sections. Using the Histogram tool, a straight line was drawn across the diameter of the fibre this produced a pixel density histogram for each fibre. However pixel value is not a measure of density. According to Beer's law (Scion J, 2002) concentration is proportional to optical density (OD). The logarithmic optical density scale, and net integral of density values for an object in an image is the proper measure for use in quantitation. By Beer's law the density of a point is the log ratio of incident light upon it and transmitted light through it:

$$OD = \text{Log}_{10}\left(\frac{I_0}{I}\right)$$

**(Equation 2)**

The CLSM does not give a flat field image. Thus, the pixel values are non linear with respect to transmission (T), which is the anti-log of the negative of OD:

$$T = 10^{-\text{O.D.}}$$

(Equation 3)

or:

$$\text{O.D.} = -\log_{10}(T) = \log_{10}\left(\frac{1}{T}\right)$$

(Equation 4)

In association with equation 3, a set of OD step tablet standards were used. The Calibrate tool on the Scion Image J software allows a transformation from pixel values directly into a scale which is linear with respect to T and into a scale which correlates to OD or concentration of stain. Thus a Look Up Table (LUT) of the OD for each muscle fibre was created which was linear to the original T pixel value created by the Histogram tool. The muscle fibres 'OD' could be broadly categorised as:

**Table 1** Look up table (LUT) of the mean optical density of 1000 muscle fibre populations.

Type <sup>†</sup>	I	IIA	IIB
O.D	111.39 ± 7.46	125.39 ± 22.50	116.38 ± 3.40

<sup>†</sup> Nomenclature according to Brooke and Kaiser (1970).

#### 2.4.7 Gel Electrophoresis

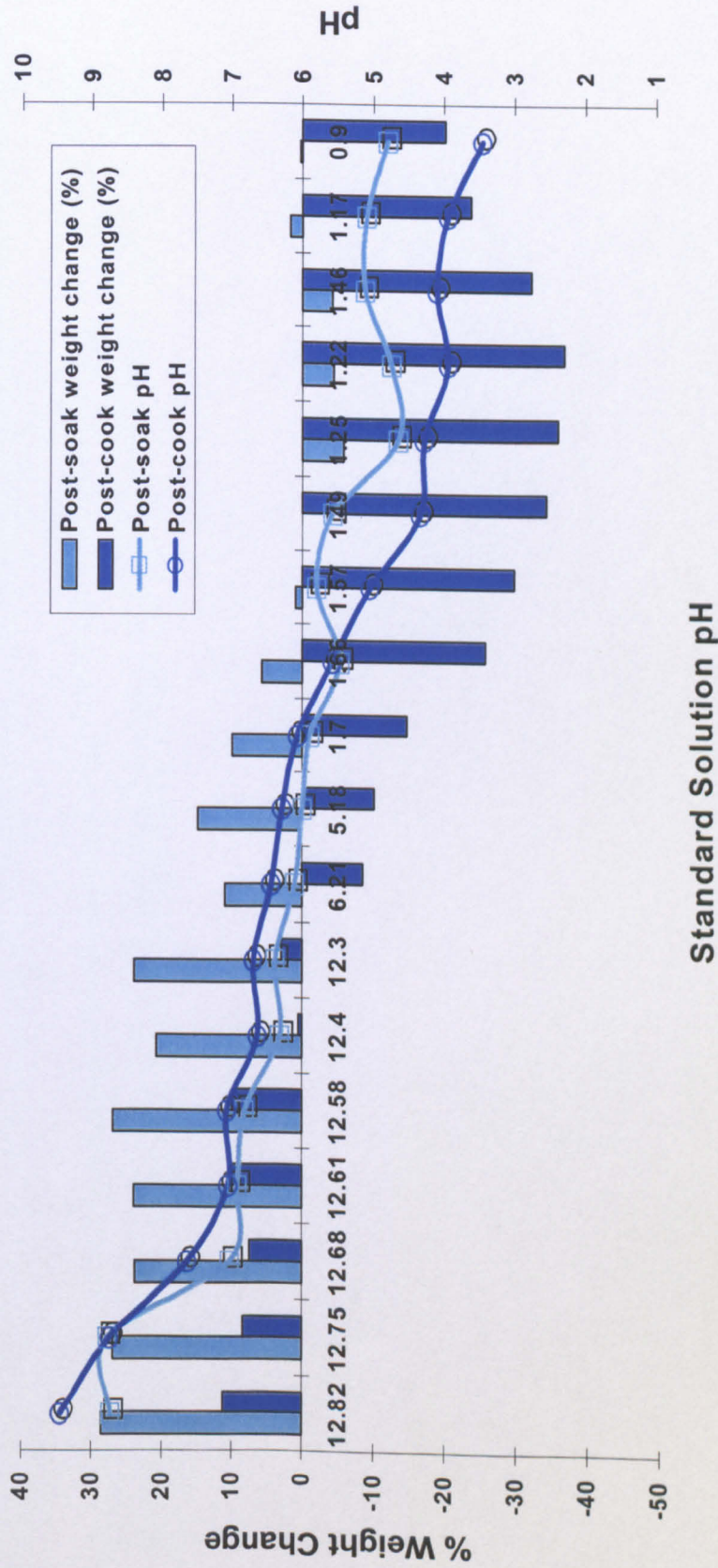
According to the method of Fairbanks *et al* (1971), linear 2 to 12 % polyacrylamide gradient gels were prepared, and 2 % polyacrylamide gels containing 0.5 % agarose (Takahashi *et al*, 1992) were used as stacking gels. Muscle samples for sodium dodecyl sulphate-polycrylamide gel electrophoresis (SDS-PAGE) were taken. For all samples, 1 g

of muscle was homogenised with 20 ml of low salt buffer (0.1 M KCl, 2 mM EGTA (ethylene glycol (bis  $\beta$ -aminoethylether)- $N,N,N',N'$ -tetraacetic acid)) 4 °C, 2mM  $MgCl_2$ , 1mM DTT (dithiothreitol), 0.001 M Tris-malate, pH 6.8). The homogenate was filtered through a stainless steel strainer (aperture: 1.0 mm), and washed six times with low salt buffer at 800 g for 10 min, and then washed four times with 5 mM tris-HCl, pH 8.0 at a speed of 3000 rpm for 10 min. Protein concentration was adjusted to 6 mg/ml, and 100  $\mu$ l of this solution was mixed with 80  $\mu$ l of electrophoresis sample buffer (8 M urea, 2 M thiourea, 3 % (w/v) SDS, 75 mM DTT, 25 mM Tris-HCl, pH 6.8 and 20  $\mu$ l of 0.1% BPB (0.1 % Bromophenol Blue, 10 % glycerine)). This solution was heated to 100 °C for 2 min and applied to the gel (45 x 50 mm x 1 mm) at 24  $\mu$ g total protein per lane. All gels were run at room temperature using a constant current of 15 mA per gel before the sample migrated into the separating gel, at which point the current was increased to 25 mA.

## 2.5 Protein functionality

Initially a method of determining the affect of different pH solutions on the soaked and cooked yield of meat was tried. This method involved soaking meat pieces (breast and thigh) with equal weights of brine solutions. The brine solutions were made up of varying concentrations of HCl, NaOH,  $H_2O$  and NaCl (full details are given in Appendix B). Although this method was too time consuming for use in subsequent trials, the results ( Figure 8and 9) were very useful as a reference for other models and later results.

Figure 8 Post-soak and post-cook breast compared to percentage weight change in different pH solutions





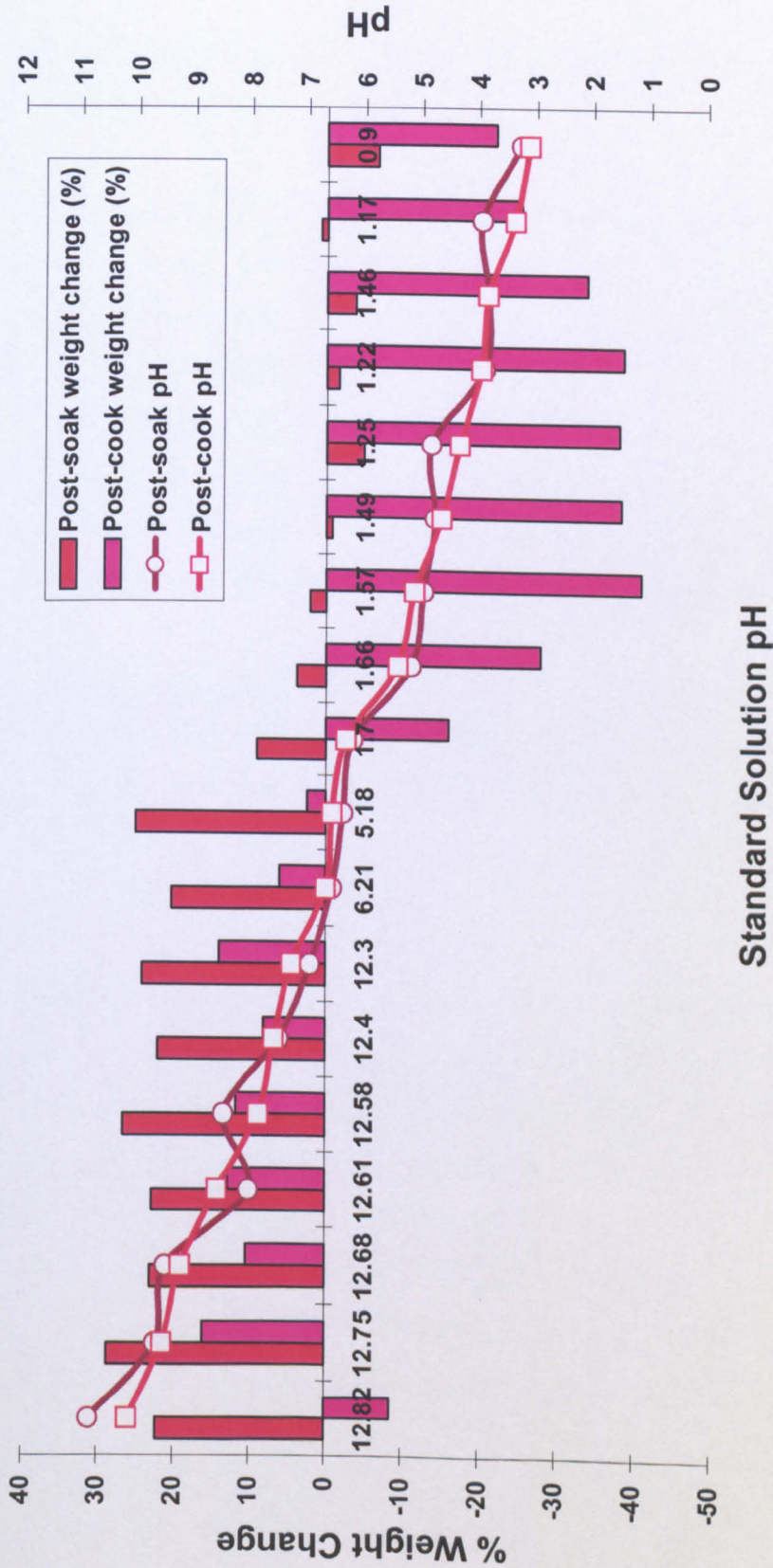


Figure 9 Post-soak and post-cook thigh pH compared to percentage weight change in different pH solutions

### 2.5.1 Whole muscle model (WMM)

As far as was practically possible protein functionality models were developed to mimic the process used in industry.

At 72 h post-mortem, the skin, all visible fat and connective tissue were removed from the surface of the left *M. pectoralis* and *M. iliobtibialis*. Using a technique adapted from Lewis *et al* (1986), a 1cm<sup>2</sup> cube of muscle was excised from the left *M. pectoralis* and *M. iliobtibialis*. Each block was cut so that its long axis ran parallel to the muscle fibre direction. The muscle cube was weighed to  $3 \pm 0.1$  g, using a top loading balance (Oertling HZ22, Germany). The pre-incubation pH of the muscle was taken at 72 h post-mortem using a glass spear probe (Philips digital pH meter, pw 9409/30, England), and placed into a 35 ml MassMold 10 polypropylene container. An equal weight of either

1. 100 % distilled water, (control); or
2. 4 % (weight for volume)(W/V) sodium chloride, NaCl, (brine 1); or
3. 1 % (W/V) sodium tripolyphosphate (anhydrous pentasodium tripolyphosphate, Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub>), (brine 2); or
4. a combination of 4 % NaCl and 1 % Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> (Brine 3)

was pipetted (Gilson, P5000, France) onto the muscle. A ratio of 1:1 (muscle : brine) was maintained due to the variation in muscle sizes. This was then refrigerated at  $3 \pm 1$  °C for 12 h, after which the muscle cubes were removed from the solutions and the post-incubation pH was taken as before. The muscle cubes were then blotted dry on a paper towel and weighed as before. The muscle cubes were then returned to the original container and solution, and a lid was fitted through which a thermocouple (Prima long, -50 to +150 °C, England) was placed. The thermocouple was placed so that the tip was in the geographic centre of the muscle sample. The polypropylene container was then immersed into a water bath set at 80 °C. Once the muscle achieved an internal temperature of  $80 \pm 1$  °C, the container was removed from the bath and placed into a

refrigerator at  $3 \pm 1$  °C for 12 h. The muscle cubes were then removed and blotted dry on a paper towel and weighed as before. The soaked and cooked sample weight were expressed as a percentage of the initial sample weight (yields).

### 2.5.2 Comminuted muscle model (CMM)

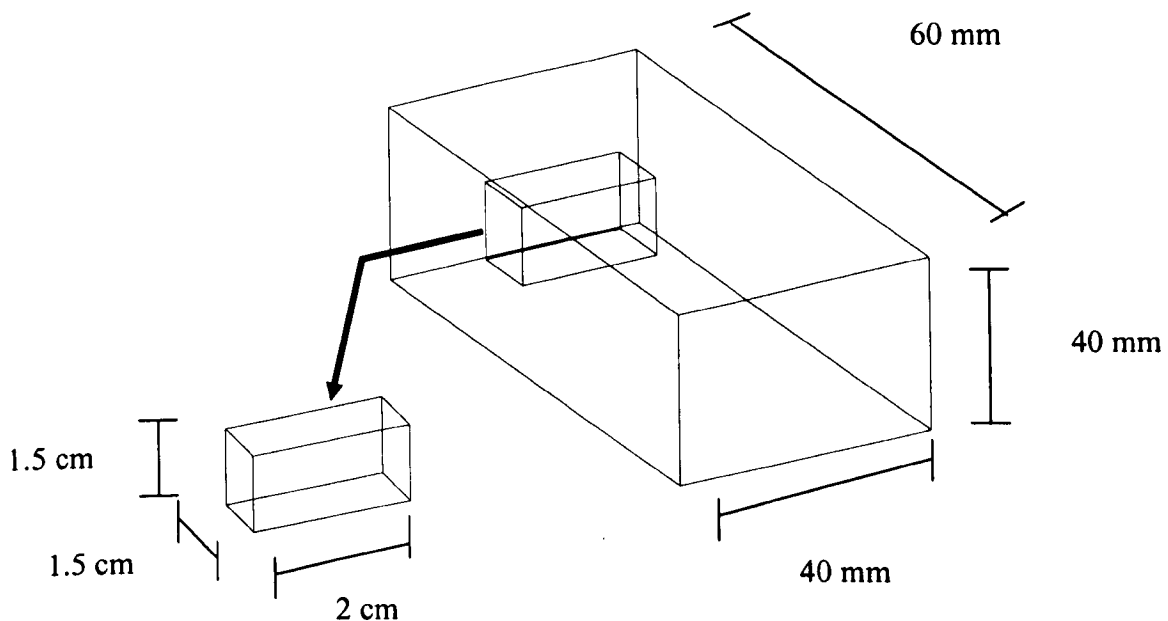
The remaining *M. pectoralis* and *M. iliobtibialis* left over from the WMM were separately minced through a 12 mm plate (Kenwood Chef, model A701A, UK). A sample was taken from the mince and placed into a mixer bowl. An equal weight of either

1. 100 % distilled water (control); or
2. 10 % (W/V) NaCl, (Brine 4); or
3. 1 % (W/V)  $\text{Na}_5\text{P}_3\text{O}_{10}$ , (Brine 5); or
4. a combination of 1 %  $\text{Na}_5\text{P}_3\text{O}_{10}$  and 10 % NaCl (Brine 6)

was added to the mince. A ratio of 4:1 (muscle : brine) by weight was maintained due to the variation in muscle weight. The mixture was then agitated using blade 3 at speed 1 for 1 min. Care was taken to ensure that the mixture did not rise above  $11 \pm 1$  °C, thus not to influence the denaturation of muscle proteins. The mixture was then removed from the bowl and added to a 200 ml polypropylene container fitted with a screw-top lid. The mixtures were then placed into a vacuum chamber (Edward High Vacuum, vacuum drier, Model EFO3, UK) and a vacuum of 760 mm of mercury was pulled to de-aerate the sample. De-aerating the mixture reduces the likelihood of textural artifacts such as layering, separation and pocket formation (Berry, 1984), which can adversely affect subsequent texture measurement. The mixture was then placed into a refrigerator at  $3 \pm 1$  °C for 12 h. A thermocouple was then fitted through the lid and a thermocouple probe was placed in to the geographic centre of the mixture. The mixture was then immersed into a water bath set at 80 °C. Once a core temperature of  $80 \pm 1$  °C was achieved, the mixture was removed from the water bath and placed in to a refrigerator at  $3 \pm 1$  °C for 12 h. The mixture was then removed from the container, blotted dry on a paper towel and weighed as before. Only a cooked yield was available, since in its raw state the mixture

was too loose to be weighed accurately. The cooked, cooled mixture was then cut into 6 blocks 2 cm x 1.5 cm x 1.5 cm for texture measurement (Figure 10).

**Figure 10** Sampling area for CMM texture measurement.



Care was taken not to use material from the outer surface. This material had a 'crust' or 'skin' which could adversely affect subsequent texture measurement (Berry, 1984).

### 2.5.3 Oil emulsion model (OEM)

Samples were processed similarly to the CMM (Section 2.5.2). However, brine 5 was not used in accordance with Lewis *et al* (1986) because these emulsions collapsed under their own weight producing material which was not weighable. After the addition of brines 4 and 6 and agitation, the three separate mixtures were then blended (Krupps, 400 ml, blender, model 708A, Republic of Ireland) at full speed for 30 sec. Vegetable oil was added to the mixture and blended for a further 30 sec. A ratio of 2:1:2 (meat to brine to oil) by weight was maintained due to variations in muscle size. The temperature ( $11 \pm 1$  °C) of the mixture was maintained to ensure that premature denaturation of proteins did

not occur. The mixtures were then placed into a vacuum chamber and a vacuum of 760 mm of mercury was pulled to de-aerate the sample. The mixture was then placed into a refrigerator at  $3 \pm 1$  °C for 12 h. The mixture was then transferred in to a pre-weighed aluminium container (410 ml) with a perforated base (2 mm perforations) and this in turn, was placed inside a larger (500ml) pre-weighed aluminium container. An aluminium stilt lifted the inner container 10 mm from the base of the outer container. A thermocouple was placed in to the geographic centre of the mixture. The mixture was then heated in a gas fired oven (Electrolux, Picasso, UK) to a core temperature of  $80 \pm 1$  °C. The mixture was placed in a refrigerator and stored at  $3 \pm 1$  °C for 12 h. Both the inner and outer containers were weighed together. Once separated, the inner container plus mixture and the outer container plus oil and liquid were weighed separately. This produced a cooked yield that was expressed as a percentage of the initial weight. All the brine treatments for the protein functionality models can be seen in Table 2.

**Table 2** Summary of brine treatments used for protein functionality models.

<b>Name</b>	<b>Composition</b>	<b>Protein functionality model</b>
Control	100 % Distilled water	WMM, CMM and OEM
Brine 1	4 % NaCl	WMM
Brine 2	1 % Na <sub>5</sub> P <sub>3</sub> O <sub>10</sub>	WMM
Brine 3	4 % NaCl and 1 % Na <sub>5</sub> P <sub>3</sub> O <sub>10</sub>	WMM
Brine 4	10 % NaCl	CMM and OEM
Brine 5	1 % Na <sub>5</sub> P <sub>3</sub> O <sub>10</sub>	CMM and OEM
Brine 6	10 % NaCl and 1 % Na <sub>5</sub> P <sub>3</sub> O <sub>10</sub>	CMM and OEM

## 2.6 Texture analysis

Both WMM and CMM samples were subjected to texture analysis. The OEM was not used for texture analysis because most samples proved to be too flaccid for texture measurement. The WMM samples were cut using a straight edge razor to 2 cm x 1.5 cm x 1.5 cm, with the longest edge parallel to fibre direction. The texture analysis was carried out using a Leatherhead Food Research Association Texture Analyser 1000 (CNS Farnell, England) (TA 1000). According to CNS Farnell, the TA 1000 was set for a distance of 8 mm, speed 0.5 mm/sec, normal cycle. The TA 26 probe (40 mm, wire cutter) was used to produce a shear value peak load (g). WMM samples were analysed with the fibre direction perpendicular to the TA 26 probe. Each CMM sample was analysed with its longest edge perpendicular to the TA 26 probe. Results were generated in Lotus 1-2-3 and delimited for Excel 97. The results are expressed as overall mean peak load (g) to prevent the need for too many graphs.

## 2.7 Microscopic analysis

WMM, CMM and OEM samples were prepared for CLSM analysis using the following method. All samples were incubated in coplin jars at room temperature unless otherwise specified.

1. WMM, CMM and OEM samples were cut ( $0.5 \text{ cm}^3$ ) using a straight edge razor.
2. Rinse carefully with solution 1 three times then in acetone and leave for 1 h.
3. Replace acetone and leave for 1h.
4. Replace acetone with 50 % alcohol/acetone and leave for 1 h.
5. Replace with 100 % alcohol and leave for 1 h.
6. Replace with 100 % alcohol (absolute) and leave for 1 h.
7. Replace with 100 % alcohol and leave overnight.
8. Replace with (50/50) alcohol/ London resin white (medium) resin and leave for 2 h.
9. Replace with 100 % London resin white (medium) resin and leave for 2 h.

10. Replace with 100 % London resin white (medium) and leave for 2 h.
11. Replace with 100 % London resin white (medium) and leave for 12 h.
12. Place the sample in a Beem® cup in 100 % LR white and leave in oven at 60 °C.

### 2.7.1 Solution 1

Phosphate buffered saline (PBS) 75 ml distilled water, 0.148 g (10.4 mM) Na<sub>2</sub>HPO<sub>4</sub> (anhydrous), 0.043 g (3.2 mM), KH<sub>2</sub>PO<sub>4</sub> (anhydrous), 0.13 g (20 mM) NaN<sub>3</sub>, 0.9 g (150 mM) NaCl, 0.1 g, Bovine serum albumin. Bring volume up to 100 ml with distilled water. Adjust to pH 7.4.

## 2.8 Image analysis

Images per muscle sample were captured using a MRC 1000 CLSM. Using the following settings. Objective lens ∞/0.17/d, NPLAN 40x/0.65, pH2. Laser excitation red, epi detector 1, filter, 680.32 nm, iris, 34, gain 930, black level 0, Mixer 1. Images were saved as Bio Rad (PIC). Using the Z-step option in the Bio Rad software several optical slices were taken through the z-axis of the meat blocks. Once all the z-steps images were recorded they were restructured to produce a virtual 3D image of the meat block.

### 2.8.1 Imaging of resin samples

The resin blocks were placed on a microscope slide and an image was captured as a Bio-Rad Confocal (PIC). The CLSM was prepared with the following settings: Objective lens ∞/0.17/d NPLAN 100x/1.25, oil, pH3, Zoom x 8.4, 514 nm green line. Filter Blocks, 1.VHS 510 DCLP, 2. Open Block. Epi Detector 1, emission 585 long pass, iris 2.6 mm, gain (v) 1320, black Level – 55. Epi Detector 2, emission open, iris 0.9, gain (v) 770, black Level 10.

## 2.9 Statistical analysis

To check for normality, data were plotted in histograms (frequency distributions) or residual verses fitted value graphs to examine for constant variance. Fibre size and distribution data were often positively distributed (left skewed) when graphed. In this case, data were transformed to give approximately normal distributions. Methods of transformation included empirical logit, +1 log, and natural log (n). Figures listed are original means  $\pm$  standard error of the mean, or standard error of the difference, for clarity. All other data, such as pH, WMM, CMM, OEM and texture; were analysed by analysis of variance after being observed to follow normal distributions. Details on analysis of data sets are set out in each chapter. All analyses were carried out using Minitab Version 13 or Genstat 5 Release 4.2.

### 2.9.1 Muscle fibre statistics

Two types of percentages were performed: a quasi-likelihood analysis for percentages of muscle types (adding up to 100%) and a weighted analysis of variance for the other variables studied. The former analysis employs a model which accounts for the dependence between the data as well as for non-linearity and variance heterogeneity (all a consequence of fact that within muscle, percentages add up to 100 %). The latter analysis employs weights to account for different variances between generations (with the smaller variance, i.e. the larger weight, for the fourth generation). In more detail:

### 2.9.2 Analysis of percentages

Suppose that  $\gamma_{ij}$  denotes the percentage of muscle type  $j$  for animal  $i$ . Let  $100 \times \pi_{ij}$  denote the expected value of  $\gamma_{ij}$ . Arbitrarily choosing type I as a reference, the expected values are modified as:  $\log(\pi_{ij}/\pi_{i1}) = \eta_{ij} = \tau_j + c_{ij}$ . Here,  $\tau_j$  is a base-line difference between the  $j$ -th and the first muscle type and  $c_{ij}$  is a modifying linear combination of main effects and interactions of experimental factors (genotype and sex) and covairables (age). Since the  $\pi_{ij}$  to 1 over muscle types  $j = 1, 2, \dots$ . It follows that:  $\pi_{ij} = \exp(\eta_{ij}) / \sum_j \exp(c\eta_{ij})$ . It is further assumed that variance and covariances follow from:  $\text{Var}(\gamma_{ij}) = \Phi \pi_{ij} (1 - \pi_{ij})$  and



$\text{Cov}(\gamma_{ij}; \gamma_{ik}) = -\Phi\pi_{ij}\pi_{ik}$ . The unknown dispersion factor  $\Phi$  is estimated by Pearson's generalised chi-square statistic divided by its degrees of freedom. Effects in  $\eta_{ij}$  are estimated by solving the quasi-likelihood equations (McCullagh and Nelder, 1989). The model was fitted with Genstat 5 release 4.2. The results of the muscle fibre proportions are expressed as ternary diagrams.

## Chapter 3

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### Experiment 1 Effects of Genotype and Sex

#### 3.1 Introduction

Broiler chickens have been selected specifically for growth and muscle size. Broiler birds yield a higher percentage of meat and lower percentage bone compared to layer birds (Dawson and Walters, 1958). Numerous authors have presented work on the increase in body weight of broiler versus layer-type chickens (Kinizetova *et al*, 1991; Gous *et al*, 1992) and muscle weight (Swatland, 1980a; Migineishvili, 1991; Vereijken, 1992; Acar *et al*, 1993; Rémignon *et al*, 2000). Generally the broiler-type birds have heavier body weights than the layer-type at the same age and the broiler-type birds have larger and heavier breast and thigh muscles compared to the layer-type at the same age.

Genetic improvement in meat yield has focused on the accentuation of the breast because of its extensive contribution to the total and economic value of poultry meat (Moran, 1999). Vereijken (1992) used a subjective score of the breast prominence relative to the keel whereas Migineishvili (1991) used keel depth as a selection tool. Meat composition is a function of many issues. Xiong (1993) measured composition, pH and protein extractability on *M. pectoralis major* and *minor* and all the muscles of the thigh on eight broiler sources. The results revealed that there was considerable variation between genotypes, particularly in pH and protein extractability, which are commonly cited as the dominant factors influencing meat processing. The results were far from conclusive but suggested that the myosin from broiler-type birds was more readily extractable than that of the layer-type birds.

The processing of surimi (normally a fish product, made from freshwater leaching of deboned fish) from mechanically deboned layer-type chicken has generated potential interest among processors (Smith *et al*, 1998). Many authors investigated the extraction and binding properties of poultry myosin (Ball Jr and Montejano, 1984; Ball Jr, 1988;

Fronning and Niemann, 1988; Amato *et al*, 1989; Dawson, 1989; Lin *et al*, 1989; Xiong and Brekke; 1991, Xiong, 1993; Lan *et al*, 1995). The aim of this chapter was to determine what effects salts and pH would have on a comminuted product. Typically layer-type meat is underutilised and is used in low-priced products. Age makes the muscle objectionably tough, due to the formation of a high amount of heat stable collagen (Nakamura *et al*, 1975). This toughness prevents its use in whole meat food and reduces the market value (Sams, 1990; Nurmahmudi and Sams, 1997). However layer-birds are high in myofibrillar protein (Lin *et al*, 1989) and could be used in comminuted heat processed products as this would greatly reduce the apparent toughness (Sams, 1990). On the other hand, the higher level of heat stable collagen may reduce the gel forming ability of layer-type meat, since collagen blocks the protein gelation structure (Saffle and Galbreath, 1964). Xiong and Brekke (1991) found that myofibrillar gel from layer-type birds was weaker than myofibrillar gel from broiler-type birds under similar gelation conditions. Nakamura *et al* (1975) observed that NaCl added to layer-type mince and incubated at 3 °C for 12 h before heating at 80 °C obtained maximum shear force of the gel. However no attempt has been made to compare the processing properties, whole muscle water bind, comminuted muscle water bind, fat bind and texture, of broiler-type and layer-type meat. Layer-type meat which is generally tougher could be comminuted in the presence of NaCl and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> to produce a product that could be reformed to into a product that would hold added water and not be objectionably tough.

The purpose of this study is to look at the effects chicken genotype and sex on the processing functionality of the breast and thigh meat plus the underpinning science of protein functionality under different processing situations.

### 3.2 Materials and methods

**Table 3** *Experimental plan for experiment 1*

Bird number	Genotype	Age (days)	Sex	Diet*
24	Broiler-type	42	Male	Standard Management
24	Layer-type	42	Male	Standard Management
24	Broiler-type	42	Female	Standard Management
24	Layer-type	42	Female	Standard Management

\*Full dietary information is given in Appendix A.

Birds were collected and muscle samples obtained and processed as described in the General Materials and Methods. Full management details are given in Appendix A.

Means of pre-incubation pH, post-incubation pH, WWM, CMM, OEM and texture were calculated per treatment group  $\pm$  standard error, checked for normal distributions, and analysed by analysis of variance.

### 3.3 Results

#### Pre and post-incubation pH

The overall mean pre-incubation pH was 5.6 for the *M. pectoralis* and 6.0 for the *M. iliotibialis*. Neither the pre-incubation nor the post-incubation pH of the *M. pectoralis* or the *M. iliotibialis* were significantly affected by genotype or sex ( $P > 0.05$ ). However, as can be seen from Table 4 the post-incubation pH of the *M. pectoralis* and *M. iliotibialis* were significantly affected by the brine treatments ( $P < 0.001$ ).

**Table 4** Overall mean post-incubation pH  $\pm$  SE of the *M. pectoralis* and the *M. iliotibialis* as affected by brines, analysed by general analysis of variance. Different superscripts within a row denotes significant differences at  $P < 0.001$ .

	<b>Control</b> (100 % Distilled water)	<b>Brine 1</b> (4 % NaCl)	<b>Brine 2</b> (1 % Na <sub>2</sub> P <sub>2</sub> O <sub>10</sub> )	<b>Brine 3</b> (4 % NaCl and 1 % Na <sub>2</sub> P <sub>2</sub> O <sub>10</sub> )
<i>M. pectoralis</i>	5.5 $\pm$ 0.01 <sup>d</sup>	5.8 $\pm$ 0.05 <sup>b</sup>	5.7 $\pm$ 0.01 <sup>c</sup>	6.1 $\pm$ 0.2 <sup>a</sup>
<i>M. iliotibialis</i>	5.8 $\pm$ 0.01 <sup>d</sup>	5.9 $\pm$ 0.01 <sup>c</sup>	6.0 $\pm$ 0.01 <sup>b</sup>	6.1 $\pm$ 0.01 <sup>a</sup>

Compared to control, brine 1 significantly increased the pH of the *M. pectoralis* by 0.3 units, with brine 2 increasing the pH by 0.2 units and brine 3 giving the greatest increase of 0.6 units. The pH of *M. iliotibialis* was increased significantly by brine 1, brine 2 and 3 ( $P < 0.001$ ). There was no interaction of genotype, sex or brine, on any of the results described. It was noted that the *M. pectoralis* of the broiler-type birds was lighter in colour than that of the layer-type birds. Also, some of the thigh muscles were almost too hard for the pH probe to penetrate. After the incubation period all the muscle samples were swollen. Individual muscle fibres could be seen splitting and separating from top of the muscle blocks that were soaked in brines 2 and 3.

#### Whole muscle model (WMM)

There was a significant difference between the overall mean soaked yield between the *M. pectoralis* (110.2 %  $\pm$  1.1) and the *M. iliotibialis* (116.0 %  $\pm$  1.0) ( $P < 0.001$ ). The soaked yield of the *M. pectoralis* and the *M. iliotibialis* was not significantly affected by the genotype or sex ( $P > 0.05$ ). As shown in Table 5, both the *M. pectoralis* and the *M. iliotibialis* were significantly affected by the brine treatments ( $P < 0.001$ ).

**Table 5** WMM soaked yield (%)  $\pm$  SE of *M. pectoralis* and the *M. iliotibialis* as affected by brines, analysed by general analysis of variance. Different superscripts within a row denotes significant differences at  $P < 0.001$ .

	<b>Control</b> (100 % Distilled water)	<b>Brine 1</b> (4 % NaCl)	<b>Brine 2</b> (1 % Na <sub>2</sub> P <sub>2</sub> O <sub>7</sub> )	<b>Brine 3</b> (4 % NaCl and 1 % Na <sub>2</sub> P <sub>2</sub> O <sub>7</sub> )
<i>M. pectoralis</i>	92.5 $\pm$ 0.2 <sup>c</sup>	103.4 $\pm$ 0.9 <sup>b</sup>	90.2 $\pm$ 0.4 <sup>d</sup>	110.9 $\pm$ 0.4 <sup>a</sup>
<i>M. iliotibialis</i>	84.4 $\pm$ 2.4 <sup>d</sup>	100.9 $\pm$ 1.7 <sup>b</sup>	95.6 $\pm$ 2.7 <sup>c</sup>	124.0 $\pm$ 2.9 <sup>a</sup>

There was also a significant interaction between the genotype and the brine treatments for the *M. iliotibialis* ( $P < 0.001$ ) but not for the *M. pectoralis* (Table 6).

There was a significant difference between the overall mean cooked yield of the *M. pectoralis* (90.0 %  $\pm$  1.5) and the *M. iliotibialis* (101.2 %  $\pm$  2.4) ( $P < 0.001$ ). The *M. pectoralis* and the *M. iliotibialis* cooked yields were not significantly affected by any of the animal production factors. Brine treatments markedly affected both the soaked (Table 5) and the cooked yield (Table 6). Interestingly, the largest cooking losses were from the *M. pectoralis* (brine 1). Brine 3 gave the highest cooking losses for the *M. iliotibialis* and brine 2 gave very similar cooking losses for both the *M. pectoralis* and the *M. iliotibialis*.

**Table 6** The interaction of broiler and layer-type birds, *M. pectoralis* and *M. iliotibialis* as affected by brines on the WMM overall mean cooked yield (%)  $\pm$  SE analysed by general analysis of variance. Within muscle types, (*M. iliotibialis* and *M. pectoralis*) values with the same superscript do not differ significantly ( $P > 0.05$ ).

	<u><i>M.pectoralis</i></u>		<u><i>M. iliotibialis</i></u>	
	<u>Broiler-type</u>	<u>Layer-type</u>	<u>Broiler-type</u>	<u>Layer-type</u>
<b>Control</b> (100 % Distilled water)	81.1 $\pm$ 0.3 <sup>de</sup>	80.9 $\pm$ 0.4 <sup>def</sup>	106.2 $\pm$ 0.3 <sup>de</sup>	104.1 $\pm$ 0.4 <sup>efg</sup>
<b>Brine 1</b> (4 % NaCl)	92.0 $\pm$ 0.3 <sup>b</sup>	88.9 $\pm$ 1.6 <sup>c</sup>	112.3 $\pm$ 0.8 <sup>c</sup>	108.9 $\pm$ 0.3 <sup>ed</sup>
<b>Brine 2</b> (1 % Na <sub>3</sub> P <sub>3</sub> O <sub>10</sub> )	81.0 $\pm$ 0.2 <sup>def</sup>	81.5 $\pm$ 0.7 <sup>d</sup>	105.6 $\pm$ 0.2 <sup>ef</sup>	101.7 $\pm$ 0.2 <sup>fg</sup>
<b>Brine 3</b> (4 % NaCl and 1 % Na <sub>3</sub> P <sub>3</sub> O <sub>10</sub> )	106.8 $\pm$ 1.3 <sup>a</sup>	107.9 $\pm$ 0.3 <sup>a</sup>	117.4 $\pm$ 0.5 <sup>b</sup>	125.3 $\pm$ 1.0 <sup>a</sup>

## Comminuted muscle model (CMM)

There was a significant difference between the mean overall yield of the breast ( $99.29\% \pm 1.3$ ) and the thigh ( $96.14\% \pm 1.7$ ). The breast and the thigh yields were not significantly affected by genotype or sex ( $P > 0.05$ ). Generally, brine 6 resulted in the highest yield than either additive alone (Table 7).

**Table 7** CMM cooked yield (%)  $\pm$  SE of breast and thigh as affected by brines, analysed by general analysis of variance. Different superscripts within a row denotes significant differences at  $P < 0.001$ .

	<b>Control</b> (100 % Distilled water)	<b>Brine 4</b> (4 % NaCl)	<b>Brine 5</b> (1 % $\text{Na}_2\text{P}_2\text{O}_7$ )	<b>Brine 6</b> (4 % NaCl and 1 % $\text{Na}_2\text{P}_2\text{O}_7$ )
<b>Breast</b>	$92.5 \pm 0.7^d$	$95.2 \pm 1.4^c$	$103.4 \pm 0.9^b$	$110.9 \pm 0.7^a$
<b>Thigh</b>	$84.1 \pm 0.6^d$	$87.4 \pm 0.8^c$	$98.5 \pm 1.1^b$	$114.4 \pm 0.7^a$

## Oil emulsion model (OEM)

There was a significant difference between the overall mean yields of the breast ( $58.6\% \pm 1.4$ ) and thigh ( $56.1\% \pm 0.7$ ) ( $P < 0.001$ ). The yields of the breast and thigh were not affected by genotype or sex, or the interaction between genotype x sex. Only the brines had a significant effect on the yield of the breast or the thigh (Table 8).

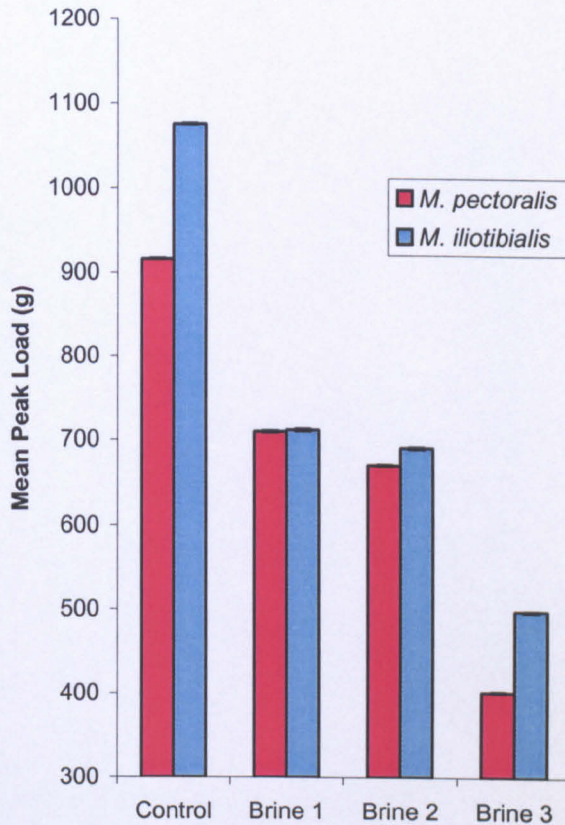


**Table 8** OEM overall mean yield (%)± SE of breast and thigh as affected by brine treatments, analysed by general analysis of variance. Different superscripts within a row denotes significant differences at  $P < 0.001$ .

	<b>Control</b> (100 % Distilled water)	<b>Brine 4</b> (10 % NaCl)	<b>Brine 6</b> (10 % NaCl and 1 % $\text{Na}_3\text{P}_2\text{O}_{10}$ )
<b>Breast</b>	48.8 ± 0.4 <sup>c</sup>	57.8 ± 0.4 <sup>b</sup>	69.1 ± 0.3 <sup>a</sup>
<b>Thigh</b>	50.4 ± 0.4 <sup>c</sup>	56.8 ± 0.2 <sup>b</sup>	61.2 ± 0.4 <sup>a</sup>

#### Whole muscle model (WMM) texture

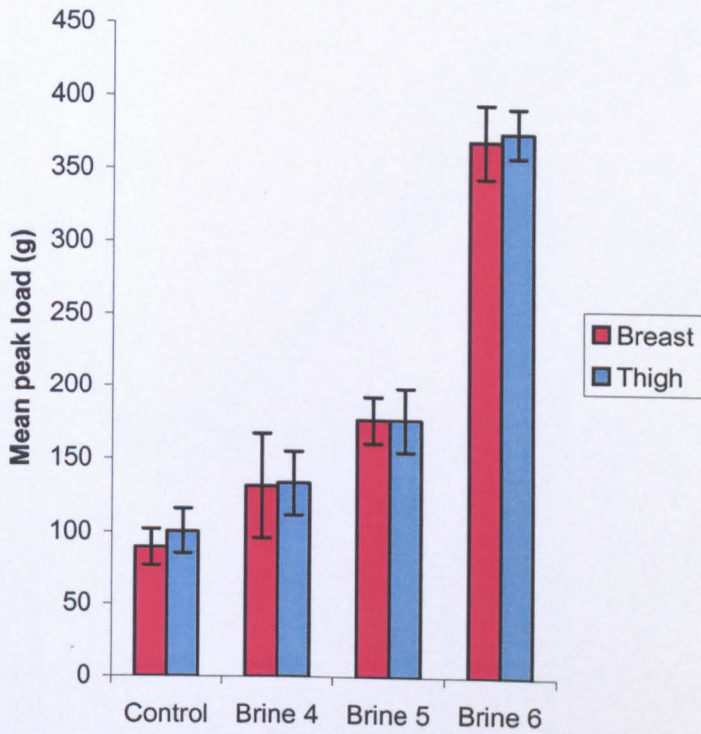
The *M. iliotibialis* gave markedly higher overall mean peak loads compared to the *M. pectoralis* ( $P < 0.001$ ). The broiler-type birds yielded the lowest overall mean peak loads (625.5 g ± 0.7) than the layer-type birds (682.3 g ± 0.9). There was no significant difference between the peak loads of male and females ( $P > 0.05$ ). Figure 11 demonstrates that brine treatments had a significant effect on the mean peak load of both the *M. pectoralis* and the *M. iliotibialis*. Brine 3 gave the lowest mean peak loads with the *M. pectoralis* and the *M. iliotibialis*.



**Figure 11** WMM mean peak loads (g) for *M. pectoralis* and the *M. iliobtibialis* as affected by brine treatments (error bars too small to see).

#### Comminuted muscle model (CMM) texture

The overall mean peak load of the breast was not significantly different from the thigh ( $P > 0.05$ ). Neither the breast nor the thigh peak loads were significantly affected by the genotype or the sex of the bird, or their interaction ( $P > 0.05$ ). The brine system significantly affected the peak load of both the breast and thigh ( $P < 0.001$ ) (Figure 12). Brine 6 gave the highest overall mean peak load for both the breast and the thigh. As can be seen from Figure 12 the control peak loads for the breast and thigh were significantly different ( $P < 0.001$ ), however brines 4, 5 and 6 produced peak loads for the breast and thigh which were not significantly different from each other ( $P > 0.05$ ).



**Figure 12** CMM overall mean peak loads (g) for breast and thigh as affected by brine treatment (with standard error bars).

Although overall mean peak load is expressed through out this thesis as the determinant of texture there were distinct differences between the raw data graph shape of the WMM and the CMM. These differences express information not only about peak loads but also about meat structure. The initial curve expresses how hard the meat is, with the curves thereafter expressing elasticity of the meat. Therefore in Figure 13, controls are much harder than brines 1, 2, and 3 and in Figure 14, brine 6 is harder and more elastic than brine 3, 4 and controls.

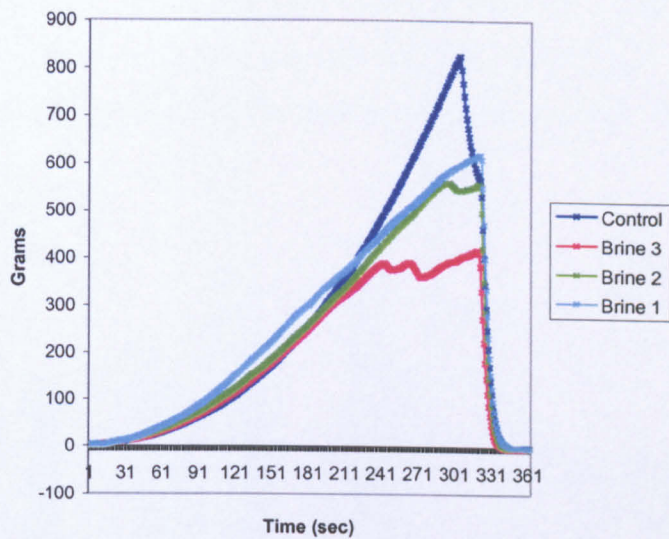


Figure 13 Typical texture curve for the WMM as affected by brine treatments

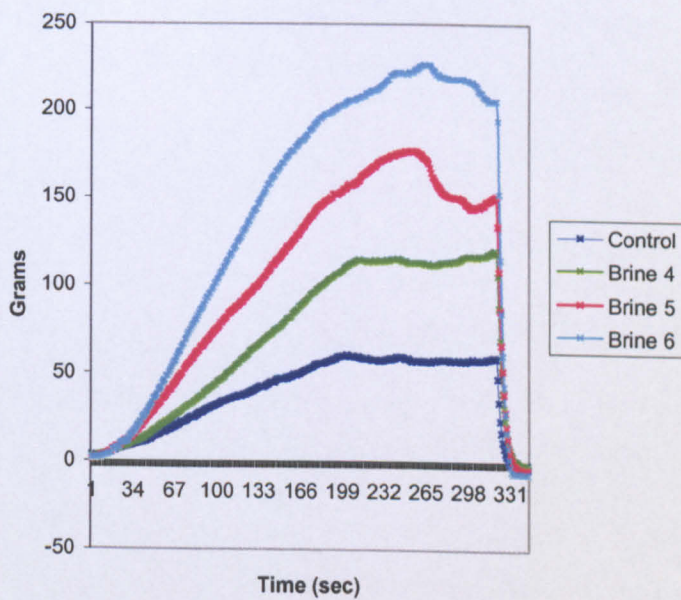
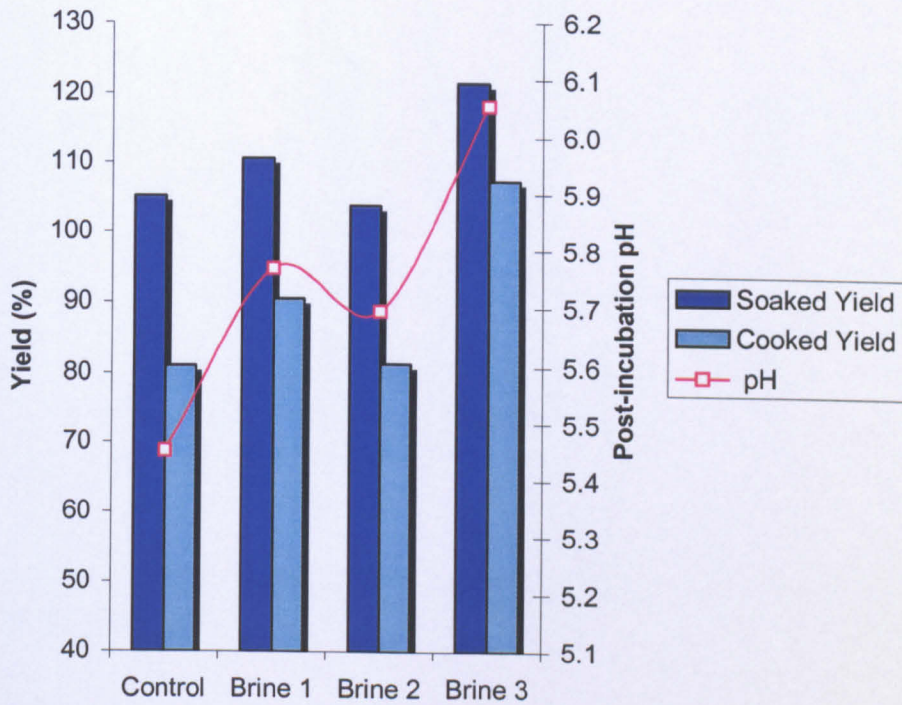
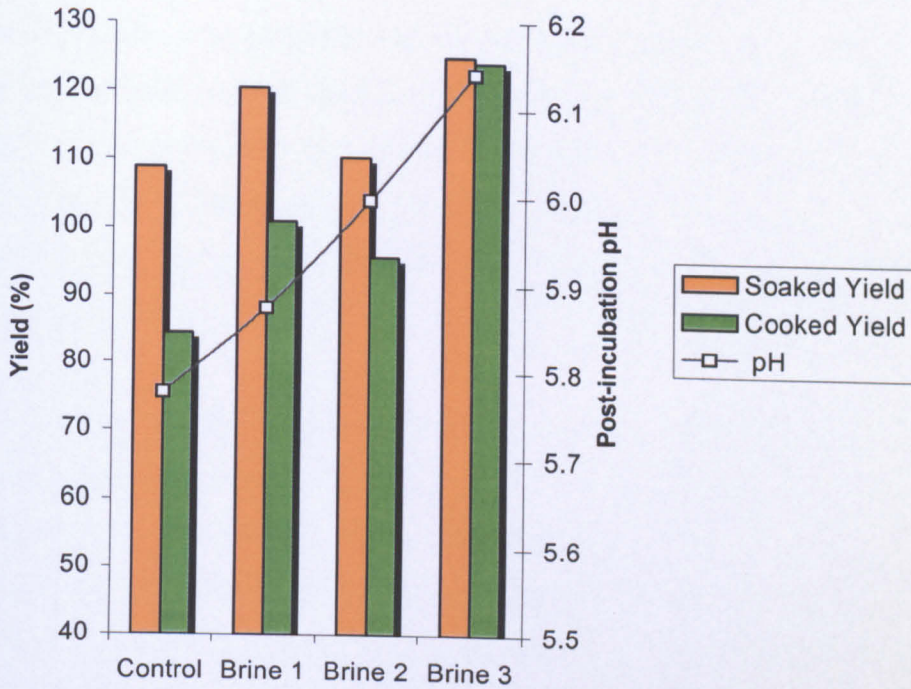


Figure 14 Typical texture curve of CMM as affected by brine treatments

There was also a clear relationship (Figure 15 and Figure 16) between the post-incubation pH and the soaked and cooked yield for both the *M. pectoralis* and the *M. iliobtibialis*. As the post-incubation pH increased so did the respective soaked and cooked yields.



**Figure 15** WMM overall mean soaked and cooked yield (%) of the *M. pectoralis* as affected by brine treatments and post-incubation pH.



**Figure 16** WMM overall mean soaked and cooked yield (%) of the *M. iliobtibialis* as affected by brine treatments and post-incubation pH.

### 3.4 Discussion

#### Pre and post-incubation pH

The objective of using two different genotypes was to determine what, if any, affect this would have on the processing capabilities of the meat. What the results show is that neither the genotype or the sex of the birds influence the pre-incubation pH, post-incubation pH, soaked, or cooked yield of either the *M. pectoralis* or the *M. iliobtibialis* regardless of whether it is a cube of muscle (WMM) or a comminute (CMM). Some differences may be expected in the WMM due to relatively intact nature of the muscle structure. It would be expected that the layer-type birds should give a lower soaked and cooked yield, (Liu *et al*, 1996; Voller *et al*, 1996), due to the interaction of the myofibril (Section 1.1.1) and the stromal network (Section 1.1.5). Certainly these structures are affected by post-mortem factors such as pH (72 h) (Section 1.5), ionic concentration of brine solutions (Section 1.3), and cooking temperature (Section 1.3.4). The post-mortem

factors have more of an effect on the processing capability than animal production factors. The post-mortem factors, particularly the brine treatments have an impact on the quaternary structure of the protein (Section 1.1.1) and it is well documented that strong acids and alkalis affect the tertiary structure of the proteins. It is probable that the effect on these structures, the structural relationship of the component polypeptides (quaternary structure) and the three-dimensional shape of the coiled or pleated polypeptides (tertiary structure) is so gross that the subtle effects of animal production factors are lost.

Part of the reason for the effectiveness of NaCl and  $\text{Na}_5\text{P}_3\text{O}_{10}$  added together or alone is that they increase the pH of the meat, which increases both the extent of myofibrillar protein extraction and the strength of the heat-set protein matrix. Although this is probably one of the most important features it does not explain all the actions. This increase in the pH would facilitate the dispersal of the sarcoplamic proteins (Section 1.1.4) in the myofibrils. This dispersal of sarcoplamic proteins could facilitate the action of NaCl on the functional proteins actin and myosin (Section 1.3.1). It is likely that the main action of NaCl is the dispersion of myofibrillar proteins; probably the A/I overlap regions rich in actomyosin. The action of phosphate can also be attributed to an increase in ionic strength in meat products (Schmidt and Trout, 1982; Trout and Schmidt, 1984; Lewis, 1986). This increase in ionic strength produces a similar action to that of NaCl. However when NaCl is combined with  $\text{Na}_5\text{P}_3\text{O}_{10}$  there is a synergistic action (Section 3.3). This synergistic action is probably as result of the increasing pH and ionic strength of the brine solution. This increase in pH and ionic strength would facilitate the precipitation of the sarcoplamic proteins from the sarcoplasm, NaCl could then disperse that myofibrillar proteins. On heating the dispersed myofibrillar proteins form a gel which retains water within the meat structure. Improved water holding capacity through the formation of myofibrillar gels is supported by many others (Velinov *et al*, 1990; Wang and Smith, 1992; Nielsen *et al*, 1995; Moiseev and Cornforth, 1997; Nowsad *et al*, 2000; Mielnik *et al*, 2002; Tsai-Fuh Tseng *et al*, 2002) and not only in poultry meat, but also in pigs (Sheard *et al*, 1999; Fernández-Martín *et al*, 2002), and beef (Boles and Swan, 2002). The formation of myofibrillar gels also increases the binding capacity of

the comminuted meat (Siegel *et al*, 1979; Nielsen *et al*, 1995; Nowsad *et al*, 2000; Mielnik *et al*, 2002).

#### Whole muscle model (WMM)

The results shown here for WMM are similar to those of Offer *et al* (1989a); Swatland and Barbut (1999a); and Nowsad *et al* (2000). These authors suggest that the addition of a NaCl solution at less than 1 M (brine 1) will increase the added water holding capacity and decrease the shear value of poultry breast and thigh. Likewise the synergistic effect of NaCl and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> at similar percentages and ionic strength has been demonstrated by Wang and Smith (1992) and Young and Braggins (1993) in beef and Sheard *et al* (1999) in pork. The addition of Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> alone (brine 2) to poultry meat has not been carried out before.

#### Comminuted muscle model (CMM)

These results suggest that the brine treatment used has a much more significant effect on the binding strength of breast and thigh comminutes than genotype and sex. These results differ with those of Xiong and Brekke (1991), who found comparatively stronger binding from layer-type birds to those from broiler-type birds. Various other researchers (Amato *et al*, 1989; Lan *et al*, 1995) have reported that gel from leg muscles had higher stress and strain values, indicating higher gel strength than the gel from breast muscle. Here, within the brine treatment controls, the layer-type meat produced significantly higher peak loads than the broiler type. However, with an increase in the pH and ionic strength of the brine, not only does the overall mean peak load increase but the differences between breast and thigh diminish. The breast and thigh peak loads of the controls were significantly different while the breast and thigh peak loads were not significantly different in brine 6. Furthermore, meat binding ability depends on many factors including pH, ionic strength, protein solubility, and muscle type as well as type and amount of extractable proteins (Lanier, 1986). As with WMM, these post-mortem factors appear to have more of an influence on the processing capability than animal production factors. Similar results have been reported by Sheard *et al* (1999), who, using pigs, showed that processing



factors (polyphosphate brines) have larger effects on tenderness than those exerted during animal production.

#### Oil emulsion model (OEM)

While not directly comparable the results here are similar to those described by Evans and Ranken (1975). In that, with added oil and additional comminution compared to CMM, the OEM tended to follow the pattern of a true emulsion (Section 1.3.3). The yields of the OEM support the theory of the action of NaCl and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> even with the addition of oil.

#### Whole muscle model (WMM) texture

The differences between the *M. pectoralis* and *M. iliobtibialis* are similar to the work presented by Ouail (1999). Some of these differences can be related to the stromal network (Section 1.1.5). However, great care was taken both with the cooking and the peak load interpretation to remove much of the stromal network influence. Bailey (1984) summarised the major events that occurred during heat processing, from 40 to 50 °C, diametrical shrinkage of the myofibrillar proteins occurred with no denaturation of endo or perimysial connective tissue. From 65 to 75 °C, thermal shrinkage of the peri and endomysium occurred and expressed much of the water from the system and tightly packed the myofibrillar proteins. At 80 °C the collagen turns to gelatin and the emphasis for texture is turned to that of the myofibrillar system. Møller (1980) refined this concept by showing that the initial deformation curve (Warner-Bratzler shear force) at 60 °C represented myofibrillar strength and the peak force represented connective tissue. When the samples were heated to a higher temperature (80 °C), which weakened the connective tissue, the initial peak became the peak force (myofibrillar) and the latter became the yield peak (weakened connective tissue). Although the results here are not from a Warner Bratzler shear test, the results are comparable (Hellyer, 2002). Thus, all samples were heat processed to over 80 ± 1 °C. Indeed, this may be exacerbated by the fact that within the *M. iliobtibialis* the connective tissue content is the major determinant of texture (Wheeler

*et al*, 2001). The results here suggest that the *M. iliotibialis* had a significantly higher mean peak load compared to the *M. pectoralis*.

### 3.5 Conclusion

The genotype and sex of birds did not play as an important role as that of brine treatments on the processing functionality of breast and thigh meat. Increasing the ionic strength of the brines resulted in an increase in soaked and cooked yields and a decrease in peak loads for the WMM, and an increase in cooked yield and an increase in peak loads for the CMM. Likewise, an increase in brine pH and ionic strength increased the yield of the OEM.

If meat is to be processed as large raw pieces of meat (WMM) the addition of brines (1,2 and 3) should improve its soaked yield, and cooked yield if the meat is to be sold cooked such as in microwave ready meals. These pieces of meat would be more tender than meat that had not been brined. If the product is to be comminuted (CMM and OEM) and restructured or reformed (nuggets or grillsteaks) then the addition of brines (4, 5 and 6) would improve the bind of the product thus reducing crumbling, cracking and breaking whilst on the processing belt, packing, transport and display. The strength of the brine could easily be altered by the meat manufacturer dependent on the desired yield of the final product.

## Chapter 4

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### Experiment 2 Effects of Genotype, Age and Sex

#### 4.1 Introduction

Koohmaraie *et al* (2002) argues that hyperplasia (increase in cell number) and hypertrophy (increase in cell size) are the determinants of muscle mass. If hyperplasia is defined as the DNA content, then it is determined by the prenatal cell proliferation and postnatal growth and development of satellite cells. Therefore, animals born with a greater number of muscle cells (e.g. double muscled cattle and broiler (breast)) have greater muscle potential (Koohmaraie *et al*, 2002). Muscle size is determined by the balance between the amount of muscle protein synthesized and the amount of muscle protein degraded. Koohmaraie states that changes in protein synthesis do not affect meat tenderness. So the speed at which an animal grows should not have too much impact on the final texture. Considering chilling, ageing, and cooking regimes, it is the state of the myofibrillar proteins which have the largest impact on meat texture with connective tissue determining background toughness. During maturation of animals' tissues, collagen becomes much more resistant to breakdown. This is not due to an increase in the number of intermolecular cross-links, indeed they may decrease (Lawrie, 1998), but to the formation of nonreducible links involving three or more chains whereby a three dimensional network is generated and a high tensile strength is developed. The nature of these mature cross-links is not yet clear, but there is evidence that hydroxyaldohistidine and pyridinoline are responsible structures. Increasing glycosylation of lysine residues may also be significant in the maturation of collagen with age (Anon, 1981). The increase of fibre diameter during maturation may be an additional contributory factor. These changes contribute to the lower solubility and higher tensile strength of connective tissue in older animals (Section 1.3.5). Although the amount of collagen in an animal may increase as animals become older, and this may have an effect on meat toughness, rapid growth of muscle fibres may dilute the relative amounts of collagen in meat. This may be particularly important when measuring the texture of broiler-type birds, which rapidly deposit larger muscle fibres compared to the layer-type birds which produce muscle more

slowly (Berri, 2000). Many reports have shown that mature collagen cross-links are more thermally stable, thus providing a rationale for toughness in cooked meat for older animals (Duance *et al*, 1977; Allain *et al*, 1978; Kopp and Valin, 1979; Light and Champion, 1984). Ledward (1984) proposed that these more stable cross-links are not only more prevalent in older animals, but that the soluble shrunken collagen may increase toughness by squeezing out moisture from the system and compressing the heat coagulated myofibrillar matrix. Immature collagen cross-links are soluble in NaCl, but mature ones are not (Richardson and Ross Murphy, 1981). Therefore NaCl is less likely to have an affect on older animals that are utilized for further processed products.

Differences in texture, and pH between chickens of different genotypes (and thus with different growth rates) may be affected by age. This is because different strains of chickens grow at different rates, and thus reach slaughter weight at different ages. To assess the effect of genotype on meat processing characteristics, different strains of birds have to be compared at the same age. Under such conditions there is no evidence for an effect of experimental or commercial selection for growth *per se* on sensory meat quality (Touraille *et al*, 1981; Delpech *et al*, 1983; Farmer *et al*, 1997). However, there is overwhelming evidence that age will affect the water holding capacity (Bendall and Restall, 1983; Wang and Smith, 1992; Swatland and Barbut, 1999b), binding properties (Voller *et al*, 1996; Nowsad *et al*, 2000, Qiao *et al*, 2001), microstructure of meat emulsions, (Voller *et al*, 1996) and the mechanical texture of the meat (Breidenstein, 1982; Berry, 1987; Liu *et al*, 1996; Voller *et al*, 1996).

The purpose of this study is to look at the effects of chicken genotype, age and sex on the processing functionality of the breast and thigh meat plus the underpinning science of protein functionality under different processing situations.

## 4.2 Materials and methods

**Table 9** *Experimental Plan for experiment 2*

Bird Number	Genotype	Age (days)	Sex	Diet*
24	Broiler-type	35	Male	Standard Management
24	Layer-type	35	Male	Standard Management
24	Broiler-type	35	Female	Standard Management
24	Layer-type	35	Female	Standard Management
24	Broiler-type	56	Male	Standard Management
24	Layer-type	56	Male	Standard Management
24	Broiler-type	56	Female	Standard Management
24	Layer-type	56	Female	Standard Management

\*Full dietary information is given in Appendix A.

Birds were collected and muscle samples obtained and processed as described in the General Materials and Methods. Full management details are given in Appendix A.

Means of pre-incubation pH, post-incubation pH, WWB, CMM, OEM and texture were calculated per treatment group  $\pm$  standard error, checked for normal distributions, and analysed by analysis of variance.

## 4.3 Results

### Pre and post-incubation pH

The overall mean *M. pectoralis* pre-incubation pH was 5.6, with the mean broiler-type 5.7, and mean layer-type 5.6. There was significant effect of genotype with the overall mean *M. iliotibialis* pre-incubation pH was 5.7, with the mean broiler-type 5.7, and the mean layer-type 5.6. There were no significant differences between the 35 and 56 day-old pre-incubation pH of the *M. pectoralis* and *M. iliotibialis* ( $P > 0.05$ ). There were no significant effects of sex on pre-incubation pH of the *M. pectoralis* or *M. iliotibialis* ( $P >$

0.05). There were no significant interactions between genotype x sex x age x brine for either the *M. pectoralis* or the *M. iliotibialis*.

There was no significant ( $P > 0.05$ ) difference between the post-incubation pH of the *M. pectoralis* and the *M. iliotibialis*. The post-incubation pH of the *M. pectoralis* was significantly affected by the brine treatment, with brine 3 giving the highest overall mean pH of 6.0, and the controls giving the lowest overall mean pH of 5.6. The post-incubation pH of the *M. iliotibialis* was also significantly affected by the brine treatment ( $P < 0.001$ ), again with brine 3 giving the highest overall pH, although brine 1 gave the lowest pH of 5.6. All brines increased the pH of the meat. Brine 3 increased the *M. pectoralis* overall mean pre-incubation pH from 5.7 to 6.0. The *M. iliotibialis* overall mean pH increased from 5.7 to 6.0. The control brines reduced the pH in some cases but generally increased the overall mean pH. Brine 1 increased the overall mean pH from 5.7 to 5.8; brine 2 increased the overall mean pH from 5.7 to 5.9. The largest increase (5.6 to 6.1) was seen with the 35 day-old broiler-type *M. pectoralis* when the meat was soaked in brine 3.

#### Whole muscle model (WMM)

There was a significant difference between the overall mean soaked yield of *M. pectoralis* ( $113.5 \% \pm 0.8$ ) and the *M. iliotibialis* ( $118.6 \% \pm 1.0$ ) ( $P < 0.001$ ). Also, the overall mean cooked yield of the *M. pectoralis* ( $86.4 \% \pm 1.2$ ) was significantly lower than the *M. iliotibialis* ( $106.2 \% \pm 1.5$ ). There were no significant differences between the broiler and the layer-type birds or a significant difference between males and females. No interaction was seen between the genotype and sex.

Both the soaked and cooked yields of the *M. pectoralis* were significantly ( $P < 0.001$ ) affected by the age of the birds. The 35 day-old birds' *M. pectoralis* gave a mean soaked yield of  $115.7 \% \pm 1.2$  and the 56 day-old birds gave a mean soaked yield of  $111.4 \% \pm 0.6$ . The *M. pectoralis* mean cooked yields were  $88.8 \% \pm 1.4$  and  $84.0 \% \pm 1.0$  for 35 and 56 day-old birds respectively. There were no significant differences between the 35 and the 56 day-old *M. iliotibialis* soaked and cooked yields. Although 35 day-old *M.*

*pectoralis* gave the highest overall mean soaked yields it also gave the lowest overall mean cooked yield of  $94.8 \% \pm 1.2$  compared to the  $99.4 \% \pm 1.0$  for the 56 day-old *M. pectoralis*. Likewise, the *M. iliotibialis* from the 35 day-old birds produced cooking losses that were lower than the 56 day-old birds, 31.9 % and 42.5 % respectively.

The soaked and cooked yield of the *M. pectoralis* and the *M. iliotibialis* was also significantly affected by the brines used to incubate the muscles ( $P < 0.001$ ) (Table 10). Predominantly, brine 3 gave the highest yields compared to all other brines. Control brines gave the highest cooking losses for the *M. pectoralis*, 32.2 % and 31.6 % for 35 day-old and 56 day-old birds. Brine 3 gave the highest cooking loss for the 35 day-old *M. iliotibialis*, although brine controls gave the highest overall cooking loss of 16 % in the 56 day-old birds.

**Table 10** WMM overall mean soaked and cooked yields (%) + SE for *M. pectoralis* and the *M. iliotibialis*, as affected by age (35 and 56 days-old) and brine treatments. Analysed by general analysis of variance. Different superscripts within a column (35 and 56 days-old) denotes significant differences at  $P < 0.001$ .

		<i>M. pectoralis</i>		<i>M. iliotibialis</i>	
Yield (%)	Age (days-old)	Soaked	Cooked	Soaked	Cooked
		35	56	35	56
<b>Control</b> (100 % Distilled water)		103.4 ± 0.7 <sup>c</sup>	103.5 ± 0.6 <sup>c</sup>	71.2 ± 1.1 <sup>f</sup>	71.9 ± 0.8 <sup>f</sup>
<b>Brine 1</b> (4 % NaCl)		120.8 ± 0.3 <sup>b</sup>	114.4 ± 0.5 <sup>c</sup>	95.8 ± 2.5 <sup>b</sup>	87.7 ± 0.9 <sup>c</sup>
<b>Brine 2</b> (1 % Na <sub>3</sub> P <sub>3</sub> O <sub>10</sub> )		113.8 ± 0.3 <sup>c</sup>	108.3 ± 0.6 <sup>d</sup>	95.8 ± 0.8 <sup>b</sup>	87.7 ± 0.5 <sup>c</sup>
<b>Brine 3</b> (4 % NaCl and 1 % Na <sub>3</sub> P <sub>3</sub> O <sub>10</sub> )		124.7 ± 0.5 <sup>a</sup>	119.2 ± 1.2 <sup>b</sup>	105.1 ± 0.8 <sup>a</sup>	98.7 ± 1.1 <sup>b</sup>
				84.7 ± 0.6 <sup>h</sup>	96.2 ± 1.2 <sup>g</sup>
				102.5 ± 0.2 <sup>c</sup>	110 ± 0.4 <sup>c</sup>
				106.6 ± 0.3 <sup>d</sup>	103.1 ± 0.1 <sup>c</sup>
				124.9 ± 0.2 <sup>a</sup>	121.5 ± 0.2 <sup>b</sup>
				80.2 ± 0.3 <sup>i</sup>	80.2 ± 0.5 <sup>i</sup>
				97.5 ± 0.8 <sup>fg</sup>	98.6 ± 0.2 <sup>fg</sup>
				98.8 ± 0.1 <sup>f</sup>	99.0 ± 0.4 <sup>f</sup>
				110.3 ± 0.4 <sup>c</sup>	110.5 ± .4 <sup>c</sup>



One observation in the *M. pectoralis* was that all brines produced a sticky exudate on the surface of the meat. After heat processing this block produced an opaque 'gel' and a cloudy, almost white, material in brines 1 and 3. Brine 3 also gave rise to a cloudy white material, and a flocculent precipitate was eventually formed. The *M. iliotibialis* produced similar material except that a reddish hue could be noted in all cases.

#### Comminuted muscle model (CMM)

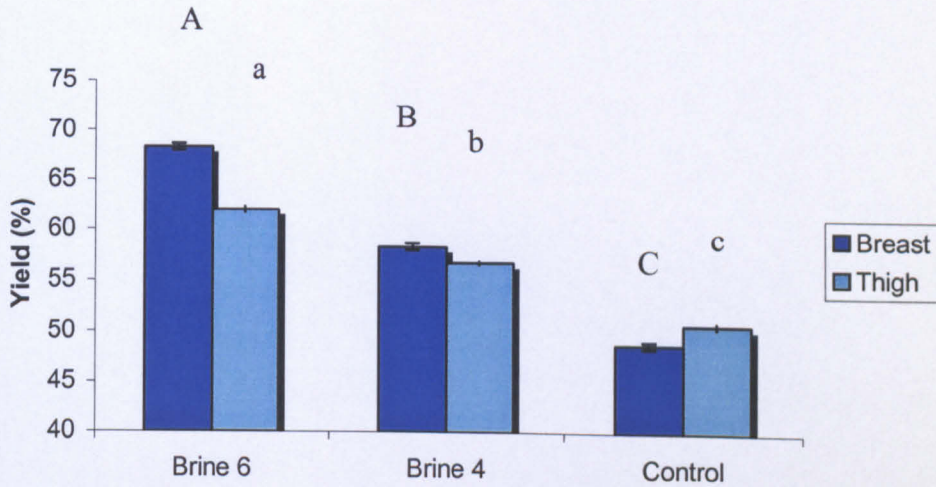
There was a significant difference between overall mean yield of the breast ( $101.4 \% \pm 0.9$ ) and the thigh ( $97.0 \% \pm 1.1$ ). Neither the breast nor the thigh cooked yields were significantly affected by genotype ( $P > 0.05$ ). Regardless of genotype the breasts from 35-day-old birds had a significantly higher cooked yield than the 56-day-old birds',  $102.5 \% \pm 1.5$  and  $100.4 \% \pm 0.6$  respectively ( $P = 0.005$ ). Table 11 shows that the mean cooked yield of the thigh was also significantly influenced by the age of the birds with the 35-day-old birds giving mean a yield of  $97.7 \% \pm 1.98$  and the 56-day-old birds showing a mean yield of  $96.4 \% \pm 0.81$  ( $P < 0.01$ ). Both the breast and the thigh cooked yields showed significant differences between brines. Generally, brine 3 resulted in the highest soaked and cooked yields than either controls, brine 1 or 2 (Table 11). When weighing the cooked meat 'emulsions' an opaque 'gel' was seen, with the breast samples the gel was loosely attached in the emulsion and appeared more watery than the gel found in the thigh emulsions. The gel found with the thigh was also darker and firmer.

**Table 11** CMM cooked yields (%)  $\pm$  SE, of the comminuted (CMM) breast and thigh as affected by age (35 and 56 day-old) and brine treatment. Analysed by general analysis of variance. Different superscripts, within muscle type (Breast and Thigh) denotes significant differences at  $P < 0.001$ .

Days	Breast		Thigh	
	<u>35</u>	<u>56</u>	<u>35</u>	<u>56</u>
<b>Control</b> (100 % Distilled water)	91.7 $\pm$ 0.4 <sup>e</sup>	92.2 $\pm$ 0.2 <sup>e</sup>	84.1 $\pm$ 0.4 <sup>h</sup>	89.2 $\pm$ 0.2 <sup>f</sup>
<b>Brine 4</b> (4 % NaCl)	107.6 $\pm$ 1.1 <sup>b</sup>	105.0 $\pm$ 0.3 <sup>c</sup>	86.7 $\pm$ 1.1 <sup>g</sup>	91.5 $\pm$ 0.7 <sup>e</sup>
<b>Brine 5</b> (1 % Na <sub>5</sub> P <sub>3</sub> O <sub>10</sub> )	93.3 $\pm$ 0.2 <sup>e</sup>	96.1 $\pm$ 0.7 <sup>d</sup>	102.5 $\pm$ 0.6 <sup>c</sup>	95.9 $\pm$ 1.3 <sup>d</sup>
<b>Brine 6</b> (4 % NaCl and 1 % Na <sub>5</sub> P <sub>3</sub> O <sub>10</sub> )	117.4 $\pm$ 0.2 <sup>a</sup>	108.2 $\pm$ 0.8 <sup>b</sup>	117.3 $\pm$ 0.5 <sup>a</sup>	109.0 $\pm$ 0.4 <sup>b</sup>

#### Oil emulsion model (OEM)

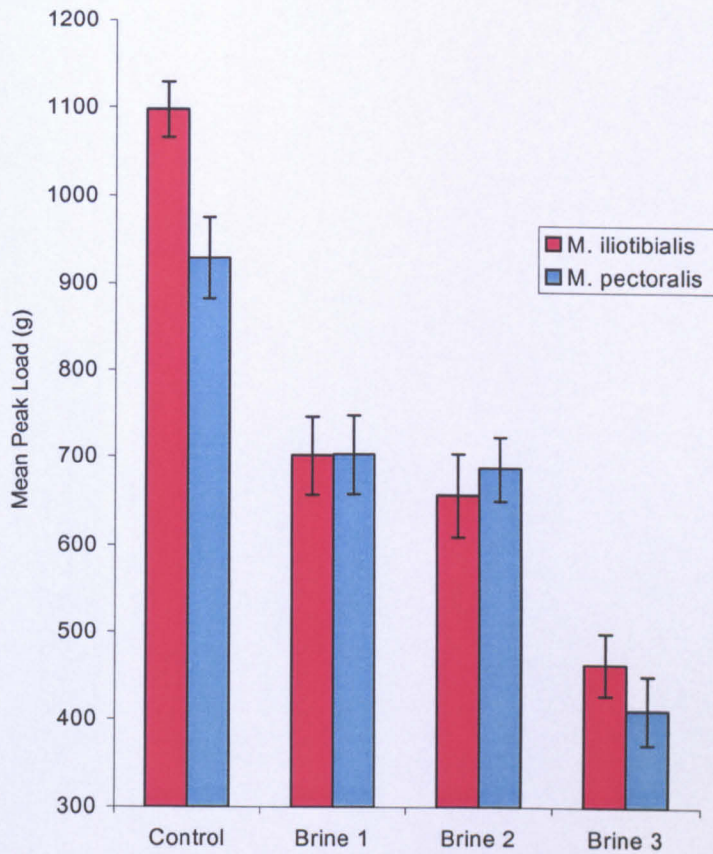
Breast (58.4 %  $\pm$  0.8) and thigh (56.4 %  $\pm$  0.5) overall mean cooked yields differed significantly. Breast and thigh yields were not significantly affected by age, genotype or sex ( $P > 0.05$ ). Also, no interactions between age x genotype x sex were significant. As can be seen in Figure 17, both the breast and the thigh cooked yields were significantly affected by the brine treatments used to incubate the muscle. Brine 6 gave the highest overall yield for both the breast and the thigh. Brine 4 gave the second largest overall yield. As with brine 6, the breast overall yield was significantly higher than the thigh yield.



**Figure 17** OEM overall yield (%) of breast and thigh as affected by brine treatment (with standard error bars). Where uppercase letters differ, significant differences at  $P = 0.001$  between breast samples. Where lowercase letters differ, significant differences at  $P = 0.001$  between thigh samples.

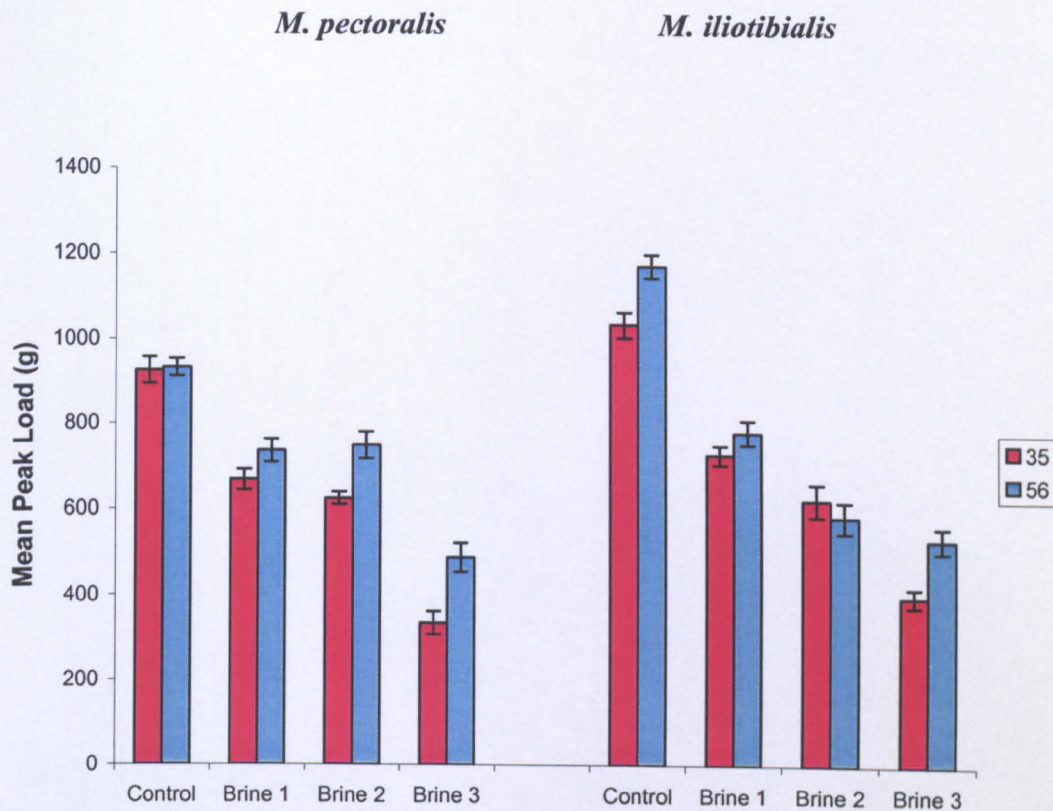
Whole muscle model (WMM) texture

The *M. iliotibialis* gave significantly higher overall mean peak loads than the *M. pectoralis* ( $P < 0.001$ ). There were no significant ( $P > 0.05$ ) differences between male and female, broiler and layer-type birds or with age within the *M. pectoralis* or the *M. iliotibialis*. Figure 18 demonstrates the significant effect of the brine on the mean peak loads of the *M. pectoralis* and the *M. iliotibialis*.



**Figure 18** WMM overall mean peak load (g) of the *M. pectoralis* and *M. iliotibialis* as affected by brine treatment (with standard error bars).

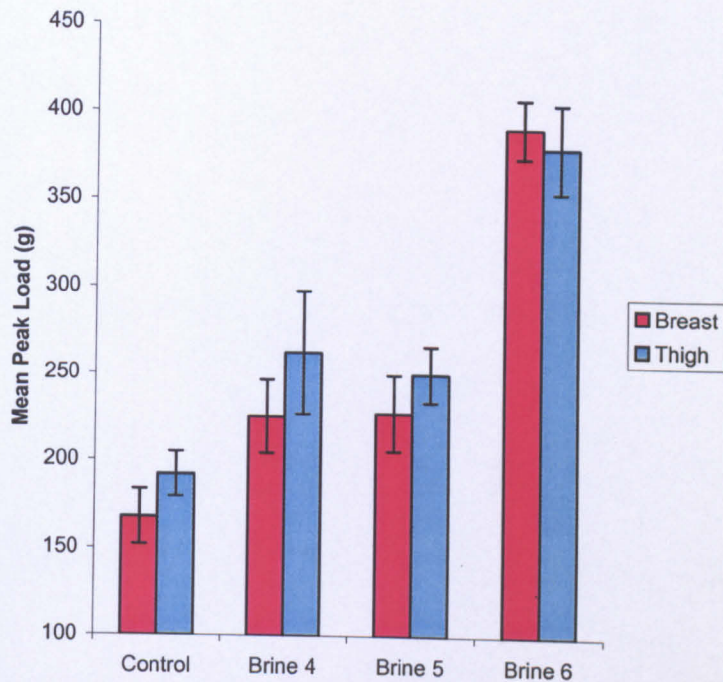
The *M. pectoralis* mean peak loads for brine 1 and 2 were not significantly different ( $P > 0.05$ ), although the *M. iliotibialis* mean peak loads for brine 1 and 2 were significantly different ( $P = 0.02$ ). There was no significant difference between the peak load of *M. pectoralis* and the *M. iliotibialis* after incubation in brine 1. Brine 3 gave significantly lower mean peak loads than any other brine treatment ( $P = 0.02$ ). There was also a significant interaction between age and brine ( $P = 0.022$ ) (Figure 19). As is shown in Figure 19, brine 1 has a much more significant affect on the 35 day-old birds rather than the 56 day-old birds for both the *M. pectoralis* and the *M. iliotibialis*.



**Figure 19** WMM overall mean peak load (g) of the *M. pectoralis* and *M. iliotibialis* as affected by age (35 ad 56 days old) and brine treatment (with standard error bars).

Comminuted muscle model (CMM) texture

There were significant differences in mean peak load between the breast ( $252 \text{ g} \pm 14$ ) and the thigh ( $270.7 \text{ g} \pm 11$ ) ( $P < 0.01$ ). With the breast, no significant differences were detected between genotypes although ( $P > 0.05$ ), there was a significant difference between ages ( $P = 0.003$ ). The 35-day-old bird gave the lowest overall mean peak load of  $242 \text{ g} \pm 15.6$  with the 56-day-old birds generating an overall mean peak load of  $262 \text{ g} \pm 23.2$ . There were no significant differences between the 35-day-old and 56-day-old birds in the thigh mean peak loads. As can be seen from Figure 20, the brines significantly affected the peak load of both the breast and the thigh ( $P < 0.01$ ).



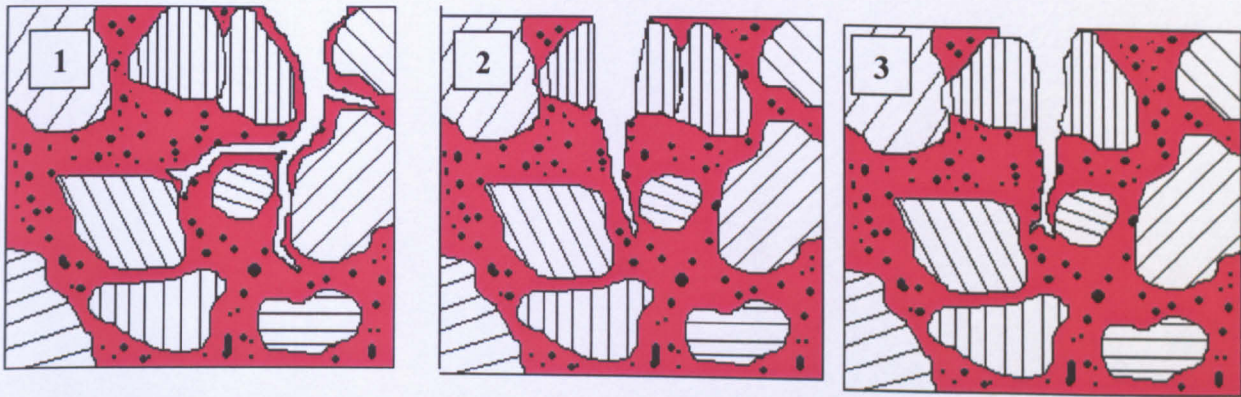
**Figure 20** CMM overall mean peak loads (g) of the breast and thigh as affected by brine treatments (with standard error bars).

Brine 6 had the greatest binding ability on both the breast and the thigh. The thigh had a consistently higher mean peak load compared to the breast with the controls, brine 4 and 5. However, with brine 6 the breast has a higher mean peak load compared to the thigh. One noteworthy observation was that three basic failure mechanisms that seemed to have occurred when the cooked CMM's were removed from the LFRA 1000 TA.

1. Either the gel surrounding the meat pieces was 'broken' (Figure 21, 1).
2. The meat pieces were partially cut with the remainder split or torn (Figure 21, 2).
3. Two opposing pieces of connective tissue seemed to be cleaved apart (Figure 21, 3).

Failure mechanism 1 was typical with thigh muscle, but particularly with layer-type birds, the gel was usually brown in colour and surrounded the meat pieces. Failure mechanism 2 was typical with broiler-type breast muscle. Failure mechanism 3 was usually observed in thigh sample but predominantly the 56-day-old layer-type birds.

**Figure 21** CMM emulsion failure mechanisms during textural measurement, the red area represents a protein gel, the black spots indicate pores and voids in the gel and the striped shapes represent meat pieces, 1 = gel failure; 2 = meat pieces splitting; 3 = cleavage of two opposing pieces of connective tissue.



#### 4.4 Discussion

##### Pre and post-incubation pH

The pre-incubation pH results are typical of the normal range (pH 5.7 to 5.6) for breast and leg muscles (pH 6.0 to 5.7) (Kijowski *et al*, 1982). As with experiment 1, an effect of genotype may have been expected with *M. pectoralis*, in that the broiler type birds should have more glycolytic-type fibres in the breast compared to the layer-type birds (Ashmore and Doerr, 1971a; Mizuno and Hikami, 1971; Suzuki, 1978; Aberle *et al*, 1979; Swatland, 1980b; Aberle and Stewart, 1983; Smith *et al*, 1993; Hoving-Bolink *et al*, 2000). As such, a distinction should have been that the broiler-type birds have a lower pre-incubation pH due to an increase in native glycogen found within the glycolytic fibres however, results here suggest the opposite. It is hard to relate this result directly to fibre types and proportions without knowledge of what the proportions of glycolytic to anaerobic fibres were in the *M. pectoralis* it. Broiler-type birds produce heavier and thicker breasts (Swatland, 1980a; Migineishvili, 1991; Vereijken, 1992; Acar *et al*, 1993; Rémignon *et al*, 2000) which would maintain a high temperature post-mortem, when compared to layer-type breast. This would result in a more rapid utilisation of ATP and attain a higher ultimate pH (72 h). This phenomenon is termed 'heat' or 'alkaline' rigor and has similar properties as pale soft and exudative (PSE) meat found in pork meat. PSE

meat is characterized by a low water-holding capacity, loose muscle structure (soft texture) and physically disrupted fibres (Lyon and Buhr, 1999) and the muscles affected usually have a high proportion of glycolytic fibres as with the *M. pectoralis*. PSE meat also has similar ultimate pH range to that of 'normal' meat.

The fact that the brines affected the post-incubation pH was anticipated and is indeed necessary for the brines to alter the processing capabilities of the meat (Lewis *et al*, 1986). The increase in pH seen in the post-incubation samples is typical (Lewis *et al*, 1986; Sheard *et al*, 1999) and relates to the ionic strength of the brine solutions which ultimately increases the pH. This increase in pH and ionic strength has previously been described in Section 3.4.

Animal production factors would not be expected to have a large effect on the post-incubation pH. Indeed, one of the reasons why NaCl and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> are used in the meat industry is to reduce 'natural' variation seen in meat pH (Lewis *et al*, 1986).

#### Whole muscle model (WMM)

The *M. pectoralis* of the 35-day-old birds gave significantly higher soaked and cooked yields in comparison to the *M. pectoralis* of the 56-day-old birds. These results can be attributed to a number of factors, not least of which is the stromal protein network (Section 1.1.5). It is well documented that as an animal matures, the Schiff base crosslinks decrease in number and are thought to convert to more stable but as yet unidentified structures. These changes contribute to the lower solubility and higher tensile strength of connective tissue in older animals. These stromal protein networks could limit the extent to which the myofibrillar proteins can swell, which regulates the water holding capacity. Thus, chronologically older animals would be expected to have a reduced soaked and cooked yield. The effect of NaCl on the collagen is also reduced with the increased in the chronological age of the animal (Richardson and Ross Murphy, 1981).



This can be seen in (Figure 19) where brine 1 (NaCl) has reduced the mean peak load much more so in the 35 day-old birds than the 56 day-old birds. One of the main effects of the  $\text{Na}_5\text{P}_3\text{O}_{10}$  on the meat would be through the increase in pH. A increase in pH can have some loosening affect on the collagen, however this is not in the range described here.

Although the meat samples were taken to  $80 \pm 1$  °C during cooking to try and limit the effect of the collagen (Voyle, 1979); (Honikel, 1998), as with experiment 1 it would appear that the stromal protein network has had an effect on the cooked yield results. Although much is known about the thermal properties of collagen, it can be difficult to isolate the effects of temperature, particularly in complex meat systems with added NaCl and  $\text{Na}_5\text{P}_3\text{O}_{10}$ .

As with experiment 1, all brine treatments significantly affected the post-incubation pH, soaked and cooked yields of both the *M. pectoralis* and the *M. iliotibialis*. One of the main actions of NaCl and  $\text{Na}_5\text{P}_3\text{O}_{10}$  either alone or in combination on meat is to move meat pH away from the isoelectric point, regardless of pre-incubation pH (Lewis *et al*, 1986; Gordon and Barbut, 1989; Gordon and Barbut, 1990; Wang and Smith, 1992).

#### Comminuted muscle model (CMM)

Comminution disrupts much of the cellular and myofibrillar structure. Thus, many of the animal production factors (genotype, age and sex) which may have had an influence on the soaked and cooked yield will be greatly reduced. If the muscle samples were not comminuted as finely, animal production factors may be seen to have more of an effect. This is why raw materials, usually thigh, with an increased connective tissue are typically comminuted to try and remove some of the natural 'variation' seen in different cuts.

Although the brines used significantly affected the post-incubation pH and the soaked and cooked yields of the *M. pectoralis* and the *M. iliotibialis* (WMM), meat with its ordered myofibrils has only a limited swelling capacity. Comminution opens up the myofibrillar structure facilitating the action of NaCl,  $\text{Na}_5\text{P}_3\text{O}_{10}$  and water, transforming

the meat from a state of limited swelling to that of unlimited swelling as explained by Hamm, (1975). The results here indicate that the addition of NaCl alone increases the yield of both the breast and the thigh the addition of NaCl and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> together increases the yield even further this is described by many authors.

#### Oil emulsion model (OEM)

The most significant effect on the OEM was that of the brines used. These results compared to that of the CMM, with brine 3 (NaCl and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub>) giving the highest yield, and the controls giving the lowest yield. Others authors (Lee, 1985; Olsson and Tornberg, 1991; Dickinson, 1992; Gordon and Barbut, 1992a; Koolmees *et al*, 1993) agree and suggest that the addition of NaCl and phosphate enhances fat stabilisation in a cooked meat emulsions (Section 1.3.3).

#### Whole muscle model (WMM) texture

Generally, the 56-day-old birds gave higher mean peak loads than the 35-day-old birds. Sarcomere length, connective tissue content, and proteolysis of myofibrils and associated proteins account for most, in not all, of the explainable variation in tenderness. Changes in protein synthesis (hyperplasia and or hypertrophy) do not affect meat tenderization (Koochmaraie *et al*, 2002). Thus according to Koochmaraie *et al* (2002) the cause of this increase in texture with age is likely to be related to the stromal proteins (Section 1.1.5). However, great care was taken to reduce the effect of stromal proteins during cooking. This said, there was a significant difference in texture between the older and the younger birds. If this difference was to be linked to the myofibrillar system, the pre-incubation pH of the older birds should have been significantly lower. As early as 1956, Howard and Lawrie found that the rate of pH fall post-mortem was inversely related to tenderness of the meat after cooking. Post-mortem muscle shortening and the development of tension occurs rapidly as the pH drops below 6.3 to 6.2 with peak tension at pH 6.0 as the muscle reaches full rigor (Newbold, 1966). When a muscle is in full rigor, the shortest sarcomere lengths are attained (1.4 to 2.0  $\mu\text{m}$  at 2 to 8 h post-mortem) and the muscle becomes

inextensible (Khan, 1974; Dunn *et al*, 1993). These results strongly suggest that the stromal proteins are influencing the texture results so that the 35-day-old birds are more tender than the 56-day-old birds. This is especially true with muscles in which connective tissue content is the major determinant such as the *M. iliotibialis* (Wheeler *et al*, 2001).

#### Comminuted muscle model (CMM) texture

Although no significant differences were found between genotype, a significant difference was found between breast and thigh muscle. Connective tissue is thought to exert an influence on the texture of restructured meat products similar to its effect on tenderness of intact muscle cuts (WWM) (Berry, 1984, 1987). Whilst Berry (1984) was referring to sensory analysis data, the textural analysis results here seems to support Berry's data. The CMM texture result seem to parallel the WMM texture results suggesting that with an increase in age there is an increase in the peak load.

Generally, the NaCl (brine 4) and NaCl and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> (brine 5) brines produced evidence of greater meat bind strength (meat binding in this case is largely determined by the shear force results). Others agree: Moore *et al* (1976) indicated that meat binding with 3.0 % NaCl was more than 100 % greater than with 1.0 % NaCl and the combination of phosphate with NaCl gave a further increase in bind strength. Indeed, much of the research evaluating the effects of NaCl levels on texture in comminuted include variations in Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub>. Commonly, the combined use of NaCl (1 to 3 %) and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> (0.25 to 0.50 %) has produced greater changes in texture than either additive alone. The results here concur suggesting a synergistic effect of NaCl and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> at a ratio of 10:1 with a brine to meat ratio of 4:1. The bind strength of the meat can be related to the myofibrillar proteins and how these are extracted. The extraction of myosin, the major functional protein, is dependent on a number of factors (Section 1.4.2). Increased bind in beef rolls (Pepper and Schmidt, 1975; Moore *et al*, 1976) and improved texture desirability in flaked and formed patties (Huffman and Cordray, 1981) and restructured pork (Schwartz and Mandigo, 1976) have been shown to result from using NaCl and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> together over using NaCl alone. The failure mechanisms for the emulsions

(Figure 21) seems similar to the work reported by Donnelly and Purslow (1987) in that the strength of the emulsion can partly be dependent upon the orientation of the meat fibres. Meat fibres, which are orientated end to end provide a strong emulsion. Meat fibres orientated side by side provide a weak emulsion and meat fibres orientated perpendicular to one another provide an intermediate emulsion.

#### **4.5 Conclusion**

There was no effect of genotype or sex on WMM, CMM or OEM. With an increase in bird age there was a decrease in soaked and cooked yield and an increase in texture in the WMM. The yields of the CMM and the OEM were not significantly affected by bird age. The bind of the CMM and OEM increased significantly with an increase in bird age.

## Chapter 5

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### Experiment 3 Effects of Genotype, Diet and Sex

#### 5.1 Introduction

The effect of dietary protein and energy concentrations on the meat quality of chickens has been the subject of a number of studies, most of which have concentrated on the sensory attributes of the meat when the energy intake was decreased from 13.5 to 9.5 MJ/kg, which resulted in the meat becoming less tender and juicy (Arafa *et al*, 1985; Ristic *et al*, 1990). Decreasing the protein content from 21 % to 13 % also leads to less tender, juicy and flavoursome meat, as well as resulting in a higher cooking loss (Ristic *et al*, 1990). The reduced tenderness of meat from birds fed low protein diets is associated with a higher content and altered composition of amino acids in the muscle collagen (Asghar *et al*, 1986). An interaction between diet and genotype has been suggested (Ricard *et al*, 1986). Indeed, when both the protein and the energy concentrations of the birds' diet are decreased, meat appears more tender in fast growing (broiler-type) chickens but tougher in slow growing (layer-type) chickens. In spite of a significant strain x diet interaction, a high energy, low protein diet generally resulted in more tender breast meat (Grey *et al*, 1986).

Although an inadequacy of any nutrient is bound to have repercussions on the amount of meat and perhaps quality only a few are of consistent concern in poultry rearing (Moran, 1999). Commercial diets without these nutrients are usually due to need rather than an error in feed formulation or mixing (Moran, 1999). These inadequacies are seldom obvious in terms of live performance, but repercussions become progressively important to yield and quality (water holding capacity, texture and colour). The breast muscle, which is economically the most important muscle, is also particularly vulnerable to these inadequacies. Reduction in energy, particularly in conjunction with a decrease in either fat or its digestibility, is unlikely to have an adverse effect when the levels of all nutrients are maintained. On the contrary, additional feed intake occurs in response to a reduction in feed energy level, and in turn, so does intake of associated nutrients (Berri, 2000).

Protein inadequacies affect the amount of meat more than its quality (Asghar *et al*, 1986). Asghar *et al*, (1986) provided 'submaintenance feeding' to growing broilers from 4 to 8 weeks of age that reduced breast and thigh muscle weights by 30 to 35 % of those fed *ad libitum*. Overall sarcoplasmic protein was reduced only in the breast as were lysosomal, microsomal and soluble fractions. This reduction in sarcoplasmic proteins may have improved the binding capacity of the meat, as sarcoplasmic proteins have been known to reduce binding strength (Section 1.4.) In another study, Roth *et al* (1990) increased dietary protein of broiler-type birds at ages corresponding to the inflection point in a way that allowed additional breast meat formation.

Most studies relate high or low protein diets to sensory attributes, no studies have tried to relate the diet to processing capabilities (soaked and cooked yield capacity in the presence of NaCl and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub>).

The purpose of this study is to look at the effects of chicken genotype, age, diet and sex on the processing functionality of the breast and thigh meat plus the underpinning science of protein functionality under different processing situations.

## 5.2 Materials and methods

**Table 12** *Experimental plan for experiment 3*

Bird Number	Genotype	Age (days)	Sex	Diet*
24	Broiler-type	42	Male	High Quality
24	Layer-type	42	Male	High Quality
24	Broiler-type	42	Female	High Quality
24	Layer-type	42	Female	High Quality
24	Broiler-type	42	Male	Low Quality
24	Layer-type	42	Male	Low Quality
24	Broiler-type	42	Female	Low Quality
24	Layer-type	42	female	Low Quality

\*Full dietary information is given in Appendix A.

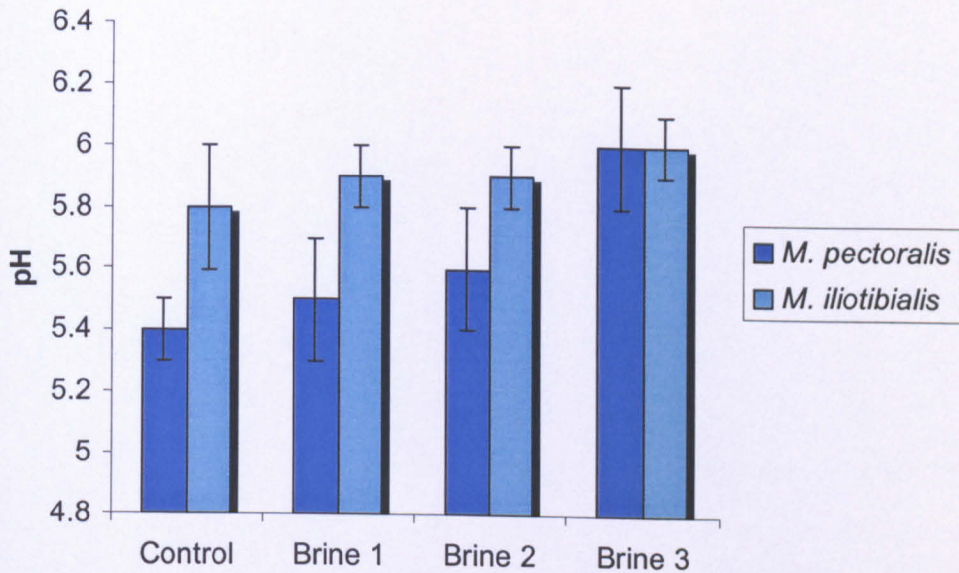
Birds were collected and muscle samples obtained and processed as described in the General Materials and Methods. Full management details are given in Appendix A.

Means of pre-incubation pH, post-incubation pH, WWB, CMM, OEM and texture were calculated per treatment group  $\pm$  standard error, checked for normal distributions, and analysed by analysis of variance.

### 5.3 Results

#### Pre and post-incubation pH

The overall pre-incubation pH was significantly lower in the *M. pectoralis* ( $5.6 \pm 0.1$ ) than the *M. iliotibialis* ( $5.9 \pm 0.2$ ) ( $P < 0.001$ ). Neither the pre-incubation pH nor the post-incubation pH of *M. pectoralis* and *M. iliotibialis* were significantly affected by genotype, sex, or diet ( $P > 0.05$ ). However, the post-incubation pH of the *M. pectoralis* and *M. iliotibialis* was significantly affected by the brine treatments ( $P < 0.001$ ). As can be seen from Figure 22, brine 3 gave the highest overall mean pre-incubation pH for both the *M. pectoralis* and *M. iliotibialis*. With the *M. pectoralis* there was no significant difference between the post-incubation pH of brine treatment 1 and 2, although there was a significant difference between the two brine treatments in the *M. iliotibialis*. It is interesting to note that even though there was a significant overall mean pre-incubation difference on pH between the *M. pectoralis* and the *M. iliotibialis*, which is reflected in through brines 1 and 2, this difference is eliminated with brine 3 (Figure 22). There was no interaction of genotype x sex x diet x brine on the post-incubation pH.



**Figure 22** Post-incubation pH of *M. pectoralis* and *M. iliotibialis* as affected by brine treatments (with standard error bars).

#### Whole muscle model (WMM)

There was significant difference between the overall mean soaked yield of the *M. pectoralis* ( $114.7\% \pm 0.2$ ) and the *M. iliotibialis* ( $118.5\% \pm 0.4$ ). The soaked yield of the *M. pectoralis* was not significantly affected by sex ( $P > 0.05$ ) but was significantly affected by genotype, diet and brine treatment ( $P < 0.001$ ) (Table 13). The *M. iliotibialis* was significantly ( $P < 0.05$ ) affected by genotype and brine with the broiler-type birds giving the highest yield.



**Table 13** WMM mean soaked and cooked yields (%)  $\pm$  SE of *M. pectoralis* as affected by diet, genotype and brine treatment. Analysed by general analysis of variance. Different superscripts within a group (soaked yield and cooked yield) denotes significant differences at  $P < 0.001$ .

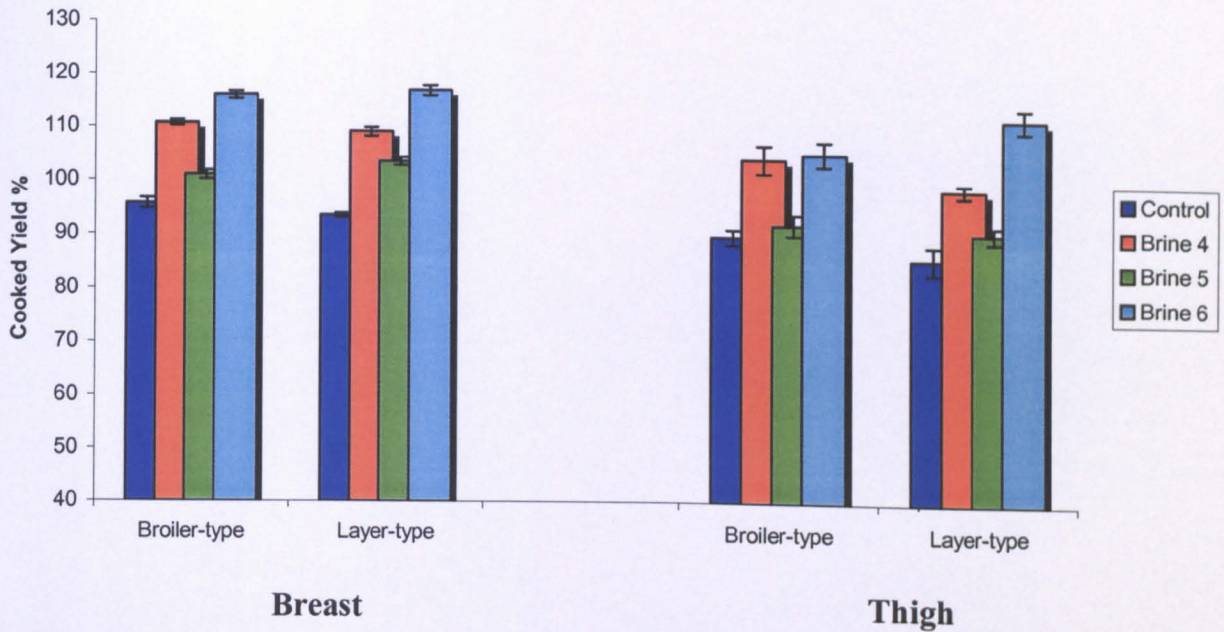
		<u>Soaked yield (%)</u>				<u>Cooked yield (%)</u>			
		<u>HQ</u>		<u>LQ</u>		<u>HQ</u>		<u>LQ</u>	
		Broiler-type	Layer-type	Broiler-type	Layer-type	Broiler-type	Layer-type	Broiler-type	Layer-type
<b><i>M. pectoralis</i></b>									
<b>Control</b> (100 % Distilled water)		108.5 $\pm$ 1.2 <sup>d</sup>	103.2 $\pm$ 1.3 <sup>e</sup>	108.3 $\pm$ 2.3 <sup>d</sup>	102.2 $\pm$ 1.0 <sup>e</sup>	84.0 $\pm$ 3.5 <sup>i</sup>	85.6 $\pm$ 3.5 <sup>hi</sup>	90.6 $\pm$ 1.7 <sup>figh</sup>	77.3 $\pm$ 2.1 <sup>j</sup>
<b>Brine 1</b> (4 % NaCl)		116.7 $\pm$ 1.5 <sup>b</sup>	118.7 $\pm$ 1.7 <sup>b</sup>	117.7 $\pm$ 1.9 <sup>b</sup>	117.9 $\pm$ 1.4 <sup>b</sup>	103.6 $\pm$ 1.6 <sup>ed</sup>	98.9 $\pm$ 2.1 <sup>de</sup>	105.1 $\pm$ 1.0 <sup>c</sup>	94.3 $\pm$ 1.4 <sup>ef</sup>
<b>Brine 2</b> (1 % Na <sub>2</sub> P <sub>2</sub> O <sub>10</sub> )		116.5 $\pm$ 1.3 <sup>b</sup>	113.2 $\pm$ 1.5 <sup>c</sup>	116.0 $\pm$ 2.0 <sup>b</sup>	110.7 $\pm$ 0.8 <sup>cd</sup>	87.1 $\pm$ 2.2 <sup>ghi</sup>	91.0 $\pm$ 1.7 <sup>fg</sup>	92.8 $\pm$ 0.8 <sup>f</sup>	93.3 $\pm$ 1.9 <sup>f</sup>
<b>Brine 3</b> (4 % NaCl and 1 % Na <sub>2</sub> P <sub>2</sub> O <sub>10</sub> )		122.7 $\pm$ 0.8 <sup>a</sup>	121.9 $\pm$ 1.6 <sup>a</sup>	122.8 $\pm$ 2.1 <sup>a</sup>	117.5 $\pm$ 1.9 <sup>b</sup>	121.2 $\pm$ 1.6 <sup>a</sup>	111.3 $\pm$ 2.2 <sup>b</sup>	112.7 $\pm$ 0.7 <sup>b</sup>	113.2 $\pm$ 3.3 <sup>b</sup>

The cooked yield of the *M. pectoralis* was significantly ( $P < 0.05$ ) affected by sex and genotype, with male broiler-type birds giving the highest yield. The brine treatments gave the most significant effect ( $P < 0.001$ ) with brine 3 giving the highest cooked yield. The *M. iliotibialis* cooked yield was not significantly affected by any of the animal production factors. Brine treatments significantly affected ( $P < 0.001$ ) the cooked yield (Table 13).

#### Comminuted muscle model (CMM)

The overall mean yield of the breast ( $101.4 \% \pm 0.9$ ) and thigh ( $97.0 \% \pm 1.1$ ) differed significantly. None of the animal production factors significantly affected the yield of the breast or the thigh. Although there was a significant interaction between the brine and genotype ( $P < 0.001$ ).

The brine significantly affected the overall mean yield of the breast and thigh ( $P < 0.001$ ), (Figure 23) There was also a significant interaction between the genotype and brine. Figure 23 demonstrates that brine 6 consistently gave a significantly higher yield than any other brine. Also, that the breast consistently gave the highest yields compared to the thigh regardless of genotype. The broiler-type birds gave the highest overall mean yield for the breast and thigh compared to the layer-type birds. It was noted that the thigh cooked meat mixture was much darker in colour than that of the breast. An iridescent sheen was seen on the surface of all the emulsions with exception of the controls. The breast cooked meat mixture was much more fragmented in appearance than the thigh, the controls more so than any other brine treatments.



**Figure 23** CMM mean yield (%) of breast and thigh as affected by genotype (with standard error bars).

Oil emulsion model (OEM)

The yields for the breast and thigh did not differ significantly ( $P > 0.05$ ). Animal production factors did not have a significant affect on the yield of either the breast or thigh. The brine treatments used gave significantly different results, with the controls giving the lowest yield of  $48.7 \% \pm 0.2$ , then brine 4 with  $58.0 \% \pm 0.3$ , and brine 6 giving the highest overall yield of  $67.9 \% \pm 0.2$  for the breast. The thigh was also significantly affected by brines, again with yields of  $56.9 \% \pm 0.2$ ,  $56.9 \% \pm 0.1$  and  $61.6 \% \pm 0.2$  for controls, brine 4 and brine 6 respectively. There was a significant ( $P = 0.026$ ) interaction between the genotype x sex x brines x diets (Table 14).

**Table 14 OEM overall mean yields (%)  $\pm$  SE as affected by genotype, sex, diet and brine treatment. Analysed by general analysis of variance. Within muscle groups, (breast and thigh) values with the same superscript do not differ significantly ( $P > 0.05$ ).**

Genotype	Sex	Brine*	Breast		Thigh	
			HQ	LQ	HQ	LQ
Broiler-type	Female	Control	68.5 $\pm$ 1.0 <sup>ab</sup>	67.4 $\pm$ 0.8 <sup>ab</sup>	61.2 $\pm$ 0.6 <sup>a</sup>	61.6 $\pm$ 1.0 <sup>a</sup>
Broiler-type	Female	Brine 4	58.3 $\pm$ 0.3 <sup>cd</sup>	60.4 $\pm$ 0.2 <sup>c</sup>	56.9 $\pm$ 0.1 <sup>b</sup>	56.9 $\pm$ 0.5 <sup>b</sup>
Broiler-type	Female	Brine 6	48.3 $\pm$ 0.7 <sup>f</sup>	48.5 $\pm$ 0.8 <sup>f</sup>	56.7 $\pm$ 0.5 <sup>b</sup>	57.7 $\pm$ 0.3 <sup>b</sup>
Broiler-type	Male	Control	66.6 $\pm$ 0.3 <sup>b</sup>	68.8 $\pm$ 0.9 <sup>a</sup>	60.9 $\pm$ 0.6 <sup>a</sup>	61.8 $\pm$ 0.8 <sup>a</sup>
Broiler-type	Male	Brine 4	57.6 $\pm$ 0.6 <sup>de</sup>	56.0 $\pm$ 0.2 <sup>c</sup>	56.9 $\pm$ 0.6 <sup>b</sup>	57.2 $\pm$ 0.4 <sup>b</sup>
Broiler-type	Male	Brine 6	48.4 $\pm$ 0.7 <sup>f</sup>	49.1 $\pm$ 0.6 <sup>f</sup>	56.3 $\pm$ 0.3 <sup>b</sup>	56.9 $\pm$ 0.6 <sup>b</sup>
Layer-type	Female	Control	68.0 $\pm$ 1.0 <sup>ab</sup>	68.1 $\pm$ 0.3 <sup>ab</sup>	62.6 $\pm$ 0.9 <sup>a</sup>	61.1 $\pm$ 0.7 <sup>a</sup>
Layer-type	Female	Brine 4	57.8 $\pm$ 0.9 <sup>de</sup>	58.3 $\pm$ 0.9 <sup>cd</sup>	57.7 $\pm$ 0.5 <sup>b</sup>	56.0 $\pm$ 0.3 <sup>b</sup>
Layer-type	Female	Brine 6	47.9 $\pm$ 0.4 <sup>f</sup>	49.0 $\pm$ 0.6 <sup>f</sup>	56.2 $\pm$ 0.3 <sup>b</sup>	56.9 $\pm$ 0.3 <sup>b</sup>
Layer-type	Male	Control	67.5 $\pm$ 0.9 <sup>ab</sup>	68.1 $\pm$ 0.3 <sup>ab</sup>	61.4 $\pm$ 0.9 <sup>a</sup>	62.0 $\pm$ 0.6 <sup>a</sup>
Layer-type	Male	Brine 4	56.7 $\pm$ 0.3 <sup>de</sup>	58.6 $\pm$ 0.9 <sup>cd</sup>	57.3 $\pm$ 0.6 <sup>b</sup>	56.3 $\pm$ 0.2 <sup>b</sup>
Layer-type	Male	Brine 6	49.3 $\pm$ 1.1 <sup>f</sup>	49.4 $\pm$ 1.1 <sup>f</sup>	56.9 $\pm$ 0.6 <sup>b</sup>	57.7 $\pm$ 0.4 <sup>b</sup>

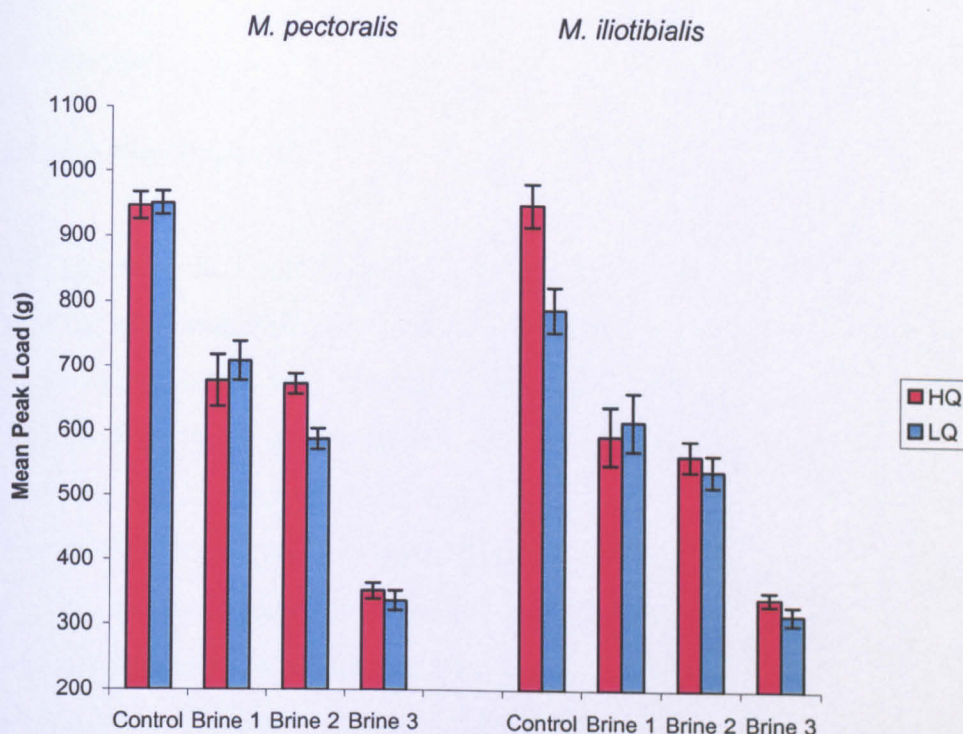
\* Control = 100 % Distilled Water; Brine 4 = (10 % NaCl); Brine 6 = (10 % NaCl and 1 % Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub>)

## Whole muscle model (WMM) texture

The overall mean peak load of the *M. pectoralis* ( $588.0 \text{ g} \pm 20.9$ ) and the *M. iliotibialis* ( $653.9 \text{ g} \pm 20.4$ ) differed significantly ( $P = 0.002$ ). The peak load of the layer-type *M. pectoralis* ( $682.3 \text{ g} \pm 29.5$ ) showed a significantly higher peak load than the broiler-type ( $625.5 \text{ g} \pm 29.4$ ) ( $P = 0.004$ ). There was also a significant interaction between genotype and sex in *M. pectoralis*, with the male layer-type giving the highest mean peak load ( $695.9 \text{ g} \pm 42.1$ ) and the male broiler-type giving the lowest mean peak load ( $602.6 \text{ g} \pm 42.2$ ). As Table 15 and Figure 24 show, significant effects of brine treatment could be seen in the *M. pectoralis* and the *M. iliotibialis* ( $P < 0.001$ ).

**Table 15** WMM overall mean peak load (g)  $\pm$  SE of the *M. pectoralis* and the *M. iliotibialis* as affected by brine treatment. Analysed by general analysis of variance. Different superscripts within a row denotes significant differences at  $P < 0.001$ .

	<b>Control</b> (100 % Distilled water)	<b>Brine 1</b> (4 % NaCl)	<b>Brine 2</b> (1 % $\text{Na}_2\text{P}_2\text{O}_7$ )	<b>Brine 3</b> (4 % NaCl and 1 % $\text{Na}_2\text{P}_2\text{O}_7$ )
<i>M. pectoralis</i>	$867.3 \pm 23.5^a$	$604.7 \pm 31.1^b$	$552.5 \pm 16.8^c$	$329.4 \pm 9.1^d$
<i>M. iliotibialis</i>	$949.2 \pm 15.6^a$	$693.1 \pm 19.1^b$	$630.2 \pm 21.1^c$	$343.1 \pm 17.8^d$



**Figure 24** WMM overall mean peak loads (g)  $\pm$  SE of breast and thigh as affected by brine treatments (with standard error bars).

Comminuted muscle model (CMM) texture

The broiler-type breast gave a significantly higher overall mean peak load ( $236.3 \text{ g} \pm 1.1$ ) than the layer-type ( $195.0 \text{ g} \pm 1.4$ ) ( $P < 0.001$ ). Neither the breast nor the thigh mean peak loads were significantly affected by sex or diet. Both the breast and the thigh were significantly affected by the brines used (Table 16).

**Table 16** CMM overall mean peak loads (g)  $\pm$  SE for breast and thigh as affected by brine treatments. Analysed by general analysis of variance within rows, different superscripts denotes significantly different at  $P < 0.001$ .

	Control (100 % Distilled water)	Brine 4 (10 % NaCl)	Brine 5 (1 % $\text{Na}_5\text{P}_3\text{O}_{10}$ )	Brine 6 (10 % NaCl and 1 % $\text{Na}_5\text{P}_3\text{O}_{10}$ )
<b>Breast</b>	$129.7 \pm 2.3^d$	$189.5 \pm 1.1^c$	$208.7 \pm 2.1^b$	$334.7 \pm 0.4^a$
<b>Thigh</b>	$125.4 \pm 1.9^d$	$168.4 \pm 1.7^c$	$181.4 \pm 2.3^b$	$315.1 \pm 0.8^a$

## 5.4 Discussion

### Pre and post-incubation pH

As with experiments 1 and 2, animal production factors did not have a significant effect on the pre-incubation pH. Both the rate and extent of post-mortem pH fall can be influenced by factors such as species, type of muscle, variability between animals, administration of drugs pre-slaughter, and the environmental temperature (Lawrie, 1998). Within the *M. pectoralis*, an increase in pre-incubation pH may be anticipated with broiler-type birds, having a predominance of glycolytic muscle fibres compared to layer-type (Ashmore and Doerr, 1971a; Ashmore *et al*, 1972). The *M. iliotibialis* has a mixed muscle fibre proportion, this proportion is largely dependent on the age of the animal as a function of the weight of the animal (Section 1.6). Thus if the animals were grown to two different chronological ages and live weights, a change in muscle morphology and proportion may reflect a change in the pre-incubation pH at 72 h. It is unlikely that the post-incubation pH would be affected by animal production factors as results from other studies (Voyle *et al* 1984; Gordon and Barbut 1989 and Gordon and Barbut 1990) suggest that brine solutions of the strengths used here (Section 1.3.1), would increase meat pH regardless of pre-incubation pH.

### Whole muscle model (WMM)

The *M. pectoralis* soaked yield was significantly affected by genotype, diet and brine treatment, but the *M. iliotibialis* was only affected by genotype and brine treatments. It seems appropriate that the *M. pectoralis* should be more sensitive to animal production factors than the *M. iliotibialis*. Over the past 50 years genetic improvement has focused on the breast muscle (*M. pectoralis major* and *minor*) (Vereijken, 1992) and most of the improvements in depth (Migineishvili, 1991) and overall yield (Moran, 1999) have come from this muscle. The difference between soaked (added water) yields of the broiler and layer-type muscle has not been extensively studied. Most of the emphasis has been on the ultimate pH (72 h) and how this relates to the water holding capacity. However the results shown here suggest that broiler meat can hold more added water due to the pre-incubation pH (72 h), due to an interaction between muscle fibre type and amount of connective tissue. Broiler-type bird breast

muscle fibres have a larger diameter than the layer-type birds (Smith, 1963). These larger muscle fibres have a diluting effect on the amount of connective tissue, and, at the same age layer-type birds have more insoluble collagen fibres (Section 1.1.5). The endomysium (Figure 1) (which is made up of collagen) acts as a mechanical restraint to swelling (Wilding *et al.* 1986). If broiler-type birds have a reduced amount and a more extendible endomysium at the same age, as layer-type birds, this would allow the broiler-type breast muscle to swell and hold more water than the layer-type breast. The high quality (HQ) diet produced the highest overall mean soaked yield in *M. pectoralis*. Although not directly comparable these results generally agree with Arafa *et al* (1985) and Ristic *et al* (1990) who suggested that reducing the protein in diets leads to less tender and juicy meat with a higher cooking loss. Asghar *et al* (1986) attributed reduced tenderness and juiciness to an increase in the insolubility of collagen. If cooking times and temperatures are consistent then meat with a larger soaked yield also results in meat with a larger cooked yield.

The soaked and cooked yield of both the *M. pectoralis* and *M. iliotibialis* were affected by brine treatments. An increase in the soaked and cooked yield can be related to the ionic strength of the brine solution. Hamm (1970) agrees stating that an increase in functionality (water binding) is directly related to the increase in ionic strength produced by the salt (NaCl). Likewise, the extent to which phosphates improve functionality is related to their effect on ionic strength and pH (Trout and Schmidt, 1984).

#### Comminuted muscle model (CMM)

The restrictive influence of the endomysium should be removed once the meat is comminuted (Offer *et al*, 1989a) therefore allowing greater swelling of the meat fibres. However, connective tissue is thought to exert an influence on the texture of restructured meat products similar to its effects on intact muscle cuts (Berry and Smith, 1985). Thus, dependent on the amount, conformation and particle size of the connective tissue and its orientation in the meat emulsion (Berry, 1984), layer-type emulsions may be liable to a reduced yield due to the contraction of collagen during cooking. Heat induced denaturation of myosin is related to species and muscle type (Wang and Smith, 1994 ; Wang and Smith, 1995; Smyth *et al*, 1998). If differences between muscles can be expressed, a difference between broiler-type breast



(predominantly glycolytic muscle fibres) and layer-type breast (a commensurate of muscle fibres) may also be expressed. During thermal processing, myofibrillar proteins in the continuous phase undergo conformational changes leading to the formation of a three dimensional gel matrix which physically entraps water and fat particles (Morrissey *et al*, 1987; Gordon and Barbut, 1992a). The broiler-type muscle may be more efficient at trapping water than the layer-type muscles due to reduced amount of connective tissue (Jolley and Purslow, 1988). As with experiment 1, brine treatments significantly increased the yield. The reason for this is discussed in experiment 1.

#### Oil emulsion model (OEM)

The results here suggest that the addition of NaCl and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> together (brine 6) has a much more significant affect than the addition of NaCl (brine 4) and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> (brine 5) alone. In turn, all of the brine treatments had a much more significant affect than any of the animal production factors. The stabilisation (retention of fat and moisture) has been attributed to many factors (Section 1.3.3) one of the most important is the extraction of myosin and the formation of heat set formation to entrap the fat and moisture. The results generally agree with those of Lee (1985), and Jolley and Purslow (1988). Gordon and Barbut (1992a) state that NaCl and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> used together produce a synergistic affect which improves the fat and moisture holding capacity of finely comminuted meats over that of meat comminutes produced with either additive alone. Finely comminuted meats tend to follow the nature of a true emulsion; the finer the emulsion (more mechanical action) the more myosin is extracted and the stronger the IPF (Section 1.3.3) physically entrapping moisture and fat in the emulsion.

#### Whole muscle model (WMM) texture

Animal production factors did not affect the overall mean peak load of the *M. iliobtibialis*. The *M. pectoralis* was more sensitive to animal production factors. The broiler-type *M. pectoralis* produced meat which was more tender than the layer-type *M. pectoralis*. Although these results reflect some of the factors affecting soaked and cooked yields, the effects of diet may not produce differences in texture large enough to be measured. The dietary variation seen in the soaked and cooked yield may only

affect the myofibrillar proteins not the stromal proteins. This is plausible as collagen, the main stromal protein, is the main determinant of mechanical textural measurements (Young and Braggins, 1993).

#### Comminuted muscle model (CMM) texture

The results here suggest that the addition of NaCl to a comminuted product improves bind strength. Studies by Moore *et al* (1976) indicate that binding strength with 3.0 % salt (NaCl) was more than 100 % greater than with 1.0 % salt (NaCl). This suggests that incorporating only 0.5 % NaCl verse no NaCl does increase the bind strength to the same extent as increasing NaCl levels from 2.0 to 2.5 %. However, a reduction in bind was noted by Cardello *et al* (1983) and Brewer *et al* (1984) with slight increases in salt (NaCl) usage at lower levels.

Generally, the combined use of NaCl (1 to 3 %) and  $\text{Na}_5\text{P}_3\text{O}_{10}$  (0.25 to 0.50 %) has produced greater changes in texture than either additive alone. Increased bind in beef rolls (Pepper and Schmidt, 1975; Moore *et al*, 1976) and improved texture desirability in flaked and formed beef patties (Huffman and Cordray, 1981) and restructured pork (Schwartz and Mandigo, 1976) have been shown to result from using NaCl and  $\text{Na}_5\text{P}_3\text{O}_{10}$  together over using either additive alone.

### 5.5 Conclusion

Breast meat had the highest soaked and cooked yields over all the models. Genotype and diet significantly affected the soaked and cooked yield of the *M. pectoralis* (WMM). The *M. iliotibialis* was significantly affected by genotype but not by diet (WMM). The layer-type birds were 'tougher' than the broiler-type birds. The CMM and the OEM were not significantly affected by animal production factors.

## Chapter 6

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### Experiment 4 Effects of Genotype, Age, Sex and Diet

#### 6.1 Introduction

Age, sex and genotype alter muscle development and meat quality (Berri, 2000). Decreasing slaughter age generally increases tenderness and juiciness (Brant and Hanson, 1962; Touraille *et al*, 1981; Sonaiya and Okeowo, 1983) the effect of age on meat quality can partly be explained by differences between genotypes with different growth rates. To assess the effect of genotype on meat quality, birds have to be compared at the same age. Under such conditions, there is no evidence for an effect of experimental or commercial selection for growth *per se* on sensory meat quality (Touraille *et al*, 1981; Delpech *et al*, 1983; Farmer *et al*, 1997). However, there is overwhelming evidence that bird age will affect the water holding capacity (Bendall and Restall, 1983; Wang and Smith, 1992; Swatland and Barbut, 1999b), binding properties (Voller-Reasonover *et al*, 1997; Nowsad *et al*, 2000; Qiao *et al*, 2001), microstructure of meat emulsions, (Voller *et al*, 1996) and the mechanical texture of the meat (Breidenstein, 1982; Berry, 1987; Liu *et al*, 1996; Voller-Reasonover *et al*, 1997). Experimental selection for increased breast yield is associated with lighter breast meat having lower drip losses (Le Bihan-Duval *et al*, 1999) and a slower rate of pH drop. Variations in the rate of fall in pH have also been reported in five commercial strains of broiler chickens (Gardzielewska *et al*, 1995). The larger and heavier breasts gave a quicker pH drop than the smaller lighter breasts.

The effect of dietary protein and energy concentrations on the meat quality of chickens has been described in a Chapter 5. An interaction between diet and genotype has been suggested (Ricard *et al*, 1986). Males grow faster and are leaner, but exhibit slightly lower breast yield than females, when tested at different ages (Houmeida *et al*, 1988), however, when comparisons are made on birds of the same age, sensory meat properties are barely affected by sex. In some studies breast meat from commercially available male broilers was slightly more tender (Simpson and Goodwin, 1975; Culioli *et al*, 1990) while in another it was claimed to be tougher

(Houmeida *et al*, 1988). It has also been observed to be juicier (Culioli *et al*, 1990); (Ristic, 1991).

The purpose of this study is to look at the effects chicken genotype, age, diet and sex on the processing functionality of the breast and thigh meat plus the underpinning science of protein functionality under different processing situations. Microscopy results demonstrating structural differences between WMM, CMM and OEM are shown in Section 6.6. Electrophoresis results demonstrating the effect of different brine systems on meat proteins are shown in Section 6.7. Muscle fibre typing and measurement results are shown in Section 6.8.

## 6.2 Materials and methods

**Table 17** *Experimental plan for experiment 4*

Bird Number	Genotype	Age (weeks)	Sex	Diet*
56	Broiler-type	1-7	Male	High Quality
56	Layer-type	1-7	Male	High Quality
56	Broiler-type	1-7	Female	High Quality
56	Layer-type	1-7	Female	High Quality
56	Broiler-type	1-7	Male	Low Quality
56	Layer-type	1-7	Male	Low Quality
56	Broiler-type	1-7	Female	Low Quality
56	Layer-type	1-7	Female	Low Quality

\* Full dietary information is given in Appendix A.

Birds were collected and muscle samples obtained and processed as described in the General Materials and Methods. Full management details are given in Appendix A.

Means of pre-incubation pH, post-incubation pH, WWB, CMM, OEM and texture were calculated per treatment group  $\pm$  standard error, checked for normal distributions, and analysed by analysis of variance. Muscle fibre types and proportions were calculated as described in Sections 2.9.1 and 2.9.2.

### 6.3 Results

#### Pre and post-incubation pH

The overall pre-incubation mean pH of the *M. pectoralis* (5.9) was significantly higher than that of the *M. iliotibialis* (5.7) ( $P < 0.001$ ). The pre-incubation pH of the *M. pectoralis* and the *M. iliotibialis* was not significantly affected by any animal production factors ( $P > 0.05$ ). The post-incubation pH was only significantly affected by the brines. Both the *M. pectoralis* and the *M. iliotibialis* yielded their highest post-incubation pH with brine 3, (5.9 and 6.0 respectively). The lowest post-incubation pH was from the controls again with the *M. iliotibialis* giving a higher mean pH. All brines increased the meat pH; the largest difference was within the broiler-type *M. pectoralis* where brine 3 increased the mean pre-incubation pH from 5.7 to a mean post-incubation pH of 6.0. The *M. iliotibialis* was least affected by brine treatments with the largest mean increase (pre-incubation pH 5.8, post-incubation pH 6.0) seen with brine 3.

#### Whole muscle model (WMM)

Genotype, sex and diets did not have an effect on the soaked and cooked yields of the *M. pectoralis* and the *M. iliotibialis*. The soaked and the cooked yield for both the *M. pectoralis* and the *M. iliotibialis* were significantly affected by the age of the birds ( $P < 0.001$ ), with both yields generally decreasing with age. The *M. iliotibialis* gave the highest overall mean soaked and cooked yield. The highest soaked yield was seen at week 1 (Table 18). Although not significantly different, the corresponding cooked yield (Table 18) was the highest seen throughout the experiment. There appeared to be a leveling off of the soaked and cooked yield at week 5, and within muscles there were no significant differences between the soaked and cooked yields at these ages. As with the pilot study and experiments 1, 2 and 3, all post-incubation meat samples appeared swollen, and a precipitate was seen on the surface of the meat and in the brine solutions. Some of the individual muscle fibres could also be seen protruding from the top of the incubated muscle blocks particularly from brines 2 and 4. A cohesive opaque gel was also seen around the cooked meat samples; the gel produced by the *M. iliotibialis* was darker in colour and was firmly bound to the meat sample.

**Table 18** WMM overall mean soaked and cooked yields (%)  $\pm$  SE of the *M. pectoralis* and the *M. iliotibialis* as affected by age, analysed by general analysis of variance. Within columns (*M. pectoralis*, soaked, *M. pectoralis*, cooked, *M. iliotibialis*, soaked, *M. iliotibialis*, cooked), values with the same superscript do not differ significantly ( $P > 0.05$ ).

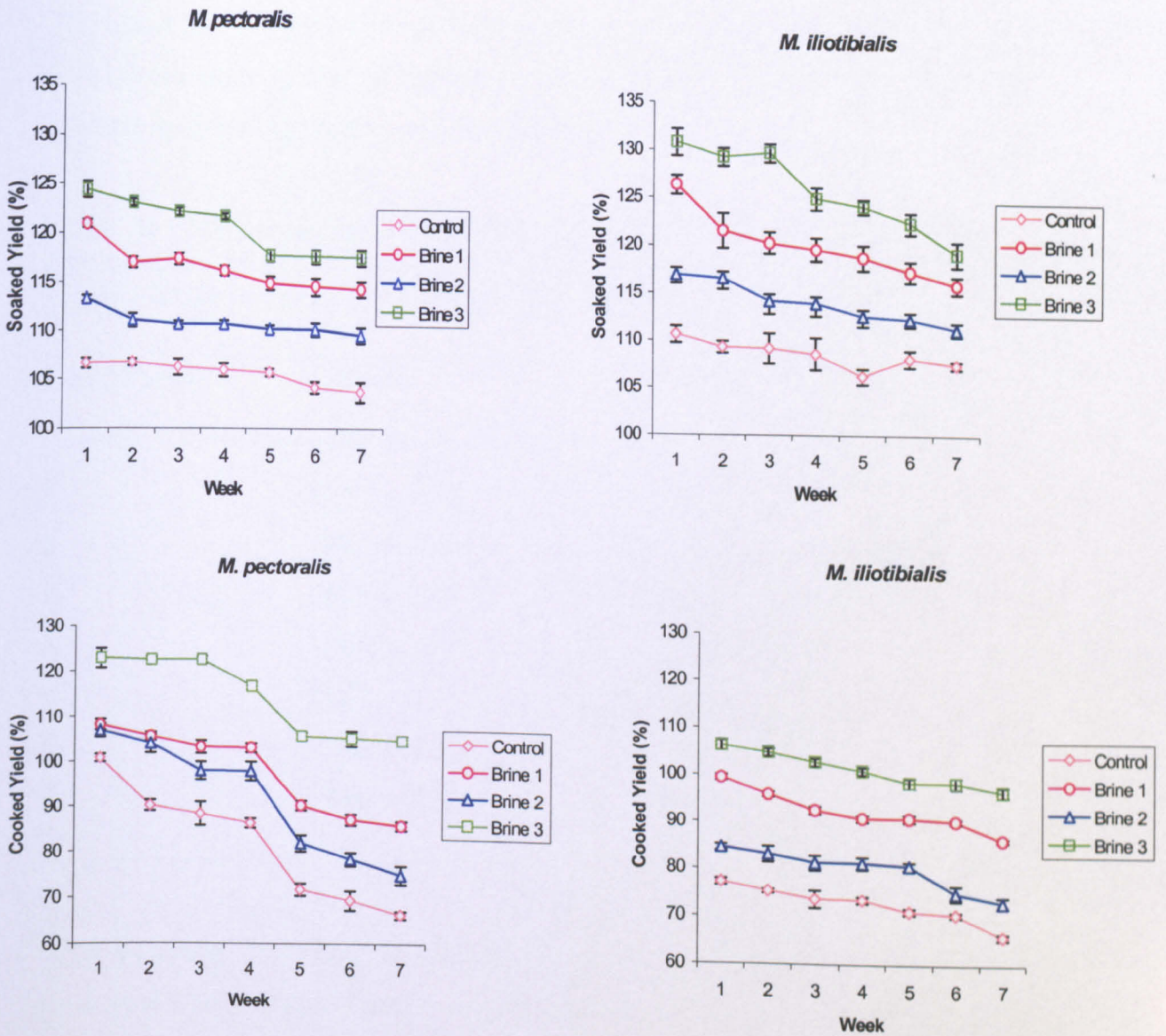
Age (Wks)	<i>M. pectoralis</i>	<i>M. pectoralis</i>	<i>M. iliotibialis</i>	<i>M. iliotibialis</i>
	Soaked	Cooked	Soaked	Cooked
1	115.5 $\pm$ 0.7 <sup>a</sup>	90.0 $\pm$ 1.0 <sup>a</sup>	120.4 $\pm$ 0.9 <sup>a</sup>	109.6 $\pm$ 1.2 <sup>a</sup>
2	114.2 $\pm$ 0.6 <sup>b</sup>	89.4 $\pm$ 1.4 <sup>a</sup>	118.9 $\pm$ 0.8 <sup>b</sup>	103.8 $\pm$ 1.3 <sup>b</sup>
3	114.1 $\pm$ 0.5 <sup>b</sup>	85.9 $\pm$ 0.9 <sup>ab</sup>	118.6 $\pm$ 0.8 <sup>b</sup>	102.8 $\pm$ 1.6 <sup>b</sup>
4	112.9 $\pm$ 0.6 <sup>c</sup>	88.8 $\pm$ 1.1 <sup>a</sup>	116.2 $\pm$ 0.7 <sup>c</sup>	102.5 $\pm$ 1.3 <sup>b</sup>
5	112.3 $\pm$ 0.4 <sup>cd</sup>	86.4 $\pm$ 0.9 <sup>ab</sup>	115.8 $\pm$ 0.4 <sup>cd</sup>	87.1 $\pm$ 1.3 <sup>c</sup>
6	112.0 $\pm$ 0.4 <sup>d</sup>	80.5 $\pm$ 1.1 <sup>c</sup>	114.7 $\pm$ 0.6 <sup>d</sup>	83.8 $\pm$ 1.3 <sup>d</sup>
7	112.0 $\pm$ 0.4 <sup>d</sup>	83.5 $\pm$ 1.1 <sup>bc</sup>	114.7 $\pm$ 0.7 <sup>d</sup>	83.9 $\pm$ 1.3 <sup>d</sup>

The brines used significantly increased both the soaked and the cooked yields of the *M. pectoralis* and the *M. iliotibialis* compared to controls ( $P < 0.001$ ). Overall, brine 3 gave the highest mean yields (Table 19) and the control gave the lowest mean yields. The *M. iliotibialis* incubated in brine 3 gave the highest soaked and cooked yields. Whilst the *M. pectoralis* soaked controls gave the lowest soaked and cooked yields.

**Table 19** WMM overall mean soaked and cooked yields (%)  $\pm$  SE of the *M. pectoralis* and the *M. iliotibialis* as affected by brine treatments, analysed by general analysis of variance. Within columns (*M. pectoralis*, soaked, *M. pectoralis*, cooked, *M. iliotibialis*, soaked, *M. iliotibialis*, cooked), different superscripts denotes significant differences at  $P < 0.001$ .

Brine	<i>M. pectoralis</i>		<i>M. iliotibialis</i>	
	Soaked	Cooked	Soaked	Cooked
<b>Control</b> (100 % Distilled water)	105.5 $\pm$ 0.1 <sup>d</sup>	72.3 $\pm$ 0.3 <sup>d</sup>	108.5 $\pm$ 0.2 <sup>d</sup>	81.7 $\pm$ 1.0 <sup>d</sup>
<b>Brine 1</b> (4 % NaCl)	110.7 $\pm$ 0.2 <sup>c</sup>	79.8 $\pm$ 0.4 <sup>c</sup>	114.0 $\pm$ 0.4 <sup>c</sup>	91.5 $\pm$ 0.7 <sup>c</sup>
<b>Brine 2</b> (1 % Na <sub>5</sub> P <sub>3</sub> O <sub>10</sub> )	116.4 $\pm$ 0.1 <sup>b</sup>	92.2 $\pm$ 0.4 <sup>b</sup>	119.9 $\pm$ 0.2 <sup>b</sup>	97.4 $\pm$ 1.0 <sup>b</sup>
<b>Brine 3</b> (4 % NaCl and 1 % Na <sub>5</sub> P <sub>3</sub> O <sub>10</sub> )	120.6 $\pm$ 0.3 <sup>a</sup>	101.1 $\pm$ 0.4 <sup>a</sup>	125.7 $\pm$ 0.5 <sup>a</sup>	114.2 $\pm$ 0.8 <sup>a</sup>

As shown in Figure 25, there was a significant interaction between age and brine ( $P < 0.001$ ), with the 1 week-old birds giving the highest overall soaked yield for the *M. pectoralis* (120.92 %  $\pm$  0.3) and the *M. iliotibialis* (130.76 %  $\pm$  1.1) when brine 3 was used. The 1-week-old birds also gave the highest overall cooked yield for the *M. pectoralis* (99.44 %  $\pm$  1.4) when brine 3 was used, but not for the *M. iliotibialis*. The lowest overall soaked yield was seen with the control brines at week 7 for both the *M. pectoralis* and the *M. iliotibialis*.



**Figure 25** WMM mean soaked and cooked yields (%) of the *M. pectoralis* and the *M. iliotibialis* as affected by brine treatment and age (with standard error bars).



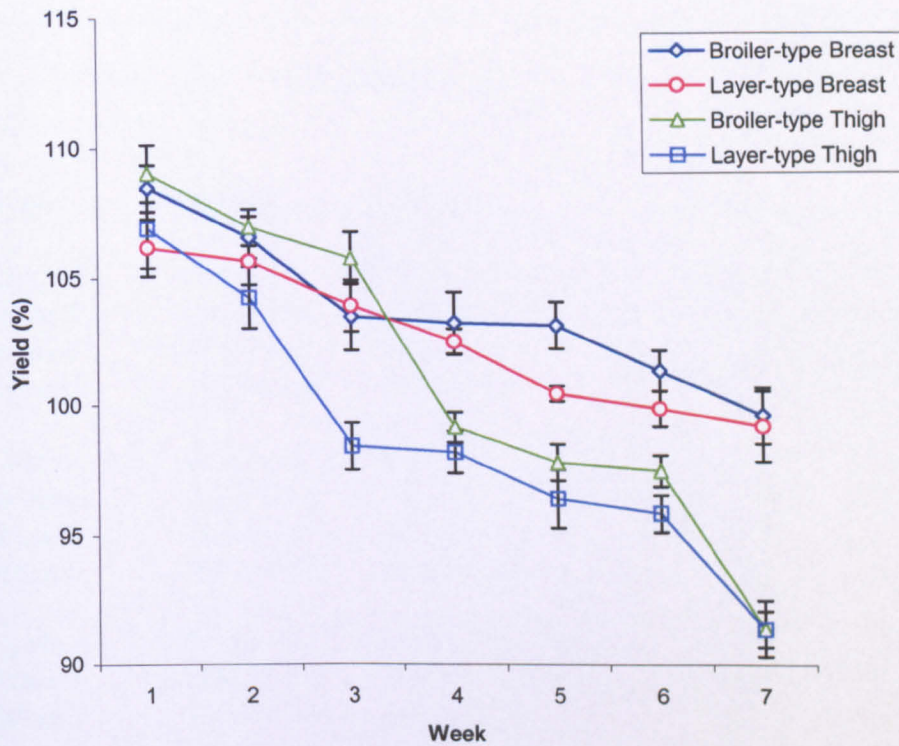
## Comminuted muscle model (CMM)

The breast and the thigh overall mean yields were significantly affected by age ( $P < 0.001$ ) with breast giving the highest overall mean yield. Table 20 shows that with an increase in animal age there was a decrease in yield.

**Table 20** CMM overall mean yields (%)  $\pm$  SE of the breast and the thigh affected by age, analysed by general analysis of variance. Within columns (Breast and Thigh), values with the same superscript do not differ significantly ( $P > 0.05$ ).

Age (Weeks)	Breast	Thigh
1	107.0 $\pm$ 0.9 <sup>a</sup>	106.9 $\pm$ 1.0 <sup>a</sup>
2	106.3 $\pm$ 0.7 <sup>a</sup>	104.9 $\pm$ 1.2 <sup>b</sup>
3	103.6 $\pm$ 1.0 <sup>b</sup>	103.7 $\pm$ 2.0 <sup>b</sup>
4	102.8 $\pm$ 1.3 <sup>c</sup>	97.9 $\pm$ 1.0 <sup>c</sup>
5	101.2 $\pm$ 1.1 <sup>d</sup>	97.4 $\pm$ 1.5 <sup>c</sup>
6	100.6 $\pm$ 0.9 <sup>de</sup>	96.9 $\pm$ 1.8 <sup>cd</sup>
7	100.0 $\pm$ 0.7 <sup>e</sup>	91.4 $\pm$ 1.0 <sup>d</sup>

When compared between genotypes, the breast of broiler-type birds (103.6 %  $\pm$  0.5) gave significantly higher overall cooked yields than the breast of the layer-type birds (102.5 %  $\pm$  0.8) ( $P < 0.001$ ). The thigh of the broiler-type birds also gave a higher yield than the layer-type birds (101.0 %  $\pm$  0.5 and 98.7 %  $\pm$  0.8 respectively). There was also a significant interaction between age x genotype for both the breast and the thigh (Figure 26). Brine treatments also gave significant differences for the breast and the thigh (Table 21).



**Figure 26** CMM overall mean yield (%) of breast and thigh as affected by genotype and age (with standard error bars).

**Table 21** CMM overall mean yield (%)  $\pm$  SE of breast and thigh as affected by brines, analysed by general analysis of variance. Different superscripts within a row denotes significant differences at  $P < 0.001$ .

	<b>Control</b> (100 % Distilled water)	<b>Brine 4</b> (10 % NaCl)	<b>Brine 5</b> (1 % $\text{Na}_5\text{P}_3\text{O}_{10}$ )	<b>Brine 6</b> (10 % NaCl and 1 % $\text{Na}_5\text{P}_3\text{O}_{10}$ )
<b>Breast</b>	93.6 $\pm$ 0.3 <sup>d</sup>	108.7 $\pm$ 0.2 <sup>b</sup>	98.0 $\pm$ 0.4 <sup>c</sup>	111.9 $\pm$ 0.3 <sup>a</sup>
<b>Thigh</b>	89.2 $\pm$ 0.6 <sup>d</sup>	104.8 $\pm$ 0.8 <sup>b</sup>	91.0 $\pm$ 0.5 <sup>c</sup>	114.5 $\pm$ 0.6 <sup>a</sup>

There was also an interaction between age x brine x genotype shown in Table 22.

**Table 22** CMM overall mean cooked yields (%)  $\pm$  SE of the breast and the thigh affected by age, brine and genotype, analysed by general analysis of variance.

Age (Wks)	Brine *	Breast		Thigh	
		Broiler	Layer	Broiler	Layer
1	Control	88.4 $\pm$ 0.1	88.5 $\pm$ 0.7	82.7 $\pm$ 0.2	81.5 $\pm$ 1.8
	Brine 4	108.8 $\pm$ 0.1	109.1 $\pm$ 0.5	110.3 $\pm$ 0.3	108.9 $\pm$ 0.2
	Brine 5	98.5 $\pm$ 0.2	97.0 $\pm$ 0.3	82.9 $\pm$ 0.3	81.9 $\pm$ 1.1
	Brine 6	116.9 $\pm$ 0.4	115.4 $\pm$ 0.3	113.9 $\pm$ 0.0	113.3 $\pm$ 0.2
2	Control	92.5 $\pm$ 0.2	92.0 $\pm$ 0.1	84.0 $\pm$ 0.2	83.2 $\pm$ 0.0
	Brine 4	107.8 $\pm$ 0.6	107.1 $\pm$ 0.1	90.4 $\pm$ 0.5	90.9 $\pm$ 0.2
	Brine 5	95.6 $\pm$ 0.3	94.5 $\pm$ 0.5	86.5 $\pm$ 0.7	87.3 $\pm$ 0.2
	Brine 6	109.7 $\pm$ 0.2	106.0 $\pm$ 0.5	104.8 $\pm$ 0.3	104.3 $\pm$ 0.1
3	Control	97.0 $\pm$ 0.3	95.8 $\pm$ 0.1	94.1 $\pm$ 0.6	83.5 $\pm$ 1.3
	Brine 4	114.1 $\pm$ 0.1	109.4 $\pm$ 0.2	122.6 $\pm$ 1.8	109.2 $\pm$ 0.7
	Brine 5	105.5 $\pm$ 0.7	103.7 $\pm$ 0.3	93.0 $\pm$ 1.4	86.8 $\pm$ 0.3
	Brine 6	117.2 $\pm$ 0.3	113.6 $\pm$ 0.6	126.4 $\pm$ 2.3	114.2 $\pm$ 0.2
4	Control	92.8 $\pm$ 0.3	91.2 $\pm$ 0.2	85.7 $\pm$ 0.4	83.0 $\pm$ 0.2
	Brine 4	111.1 $\pm$ 0.4	104.7 $\pm$ 0.6	101.8 $\pm$ 0.9	99.5 $\pm$ 0.5
	Brine 5	95.5 $\pm$ 0.6	90.9 $\pm$ 1.1	92.8 $\pm$ 0.4	85.4 $\pm$ 0.9
	Brine 6	113.5 $\pm$ 0.49	109.9 $\pm$ 0.6	116.2 $\pm$ 0.41	115.5 $\pm$ 0.5
5	Control	98.9 $\pm$ 0.2	99.2 $\pm$ 0.6	95.3 $\pm$ 1.2	95.9 $\pm$ 0.7
	Brine 4	109.9 $\pm$ 0.5	111.3 $\pm$ 0.2	112.2 $\pm$ 1.2	106.4 $\pm$ 0.7
	Brine 5	103.0 $\pm$ 0.9	101.8 $\pm$ 0.5	97.4 $\pm$ 1.2	96.5 $\pm$ 2.3
	Brine 6	114.3 $\pm$ 0.3	112.3 $\pm$ 0.5	118.0 $\pm$ 0.6	118.1 $\pm$ 1.8
6	Control	94.0 $\pm$ 0.7	94.9 $\pm$ 1.8	99.7 $\pm$ 0.1	98.4 $\pm$ 1.3
	Brine 4	110.3 $\pm$ 0.4	110.4 $\pm$ 1.0	110.1 $\pm$ 0.1	110.5 $\pm$ 0.5
	Brine 5	96.8 $\pm$ 0.7	98.3 $\pm$ 0.5	98.9 $\pm$ 0.6	99.3 $\pm$ 0.1
	Brine 6	112.8 $\pm$ 0.3	112.0 $\pm$ 0.5	119.1 $\pm$ 0.3	119.3 $\pm$ 0.2
7	Control	92.2 $\pm$ 1.0	93.7 $\pm$ 0.5	90.8 $\pm$ 0.7	90.9 $\pm$ 1.1
	Brine 4	104.7 $\pm$ 0.2	104.2 $\pm$ 0.7	97.8 $\pm$ 1.1	96.8 $\pm$ 1.2
	Brine 5	94.4 $\pm$ 0.3	97.9 $\pm$ 0.6	91.6 $\pm$ 0.6	94.8 $\pm$ 0.8
	Brine 6	107.2 $\pm$ 0.7	106.1 $\pm$ 0.7	111.1 $\pm$ 1.9	110.3 $\pm$ 1.8

\* Control = (100 % Distilled water); Brine 1 = (4 % NaCl); Brine 2 = (1 % Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub>); Brine 3 = (4 % NaCl and 1 % Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub>).

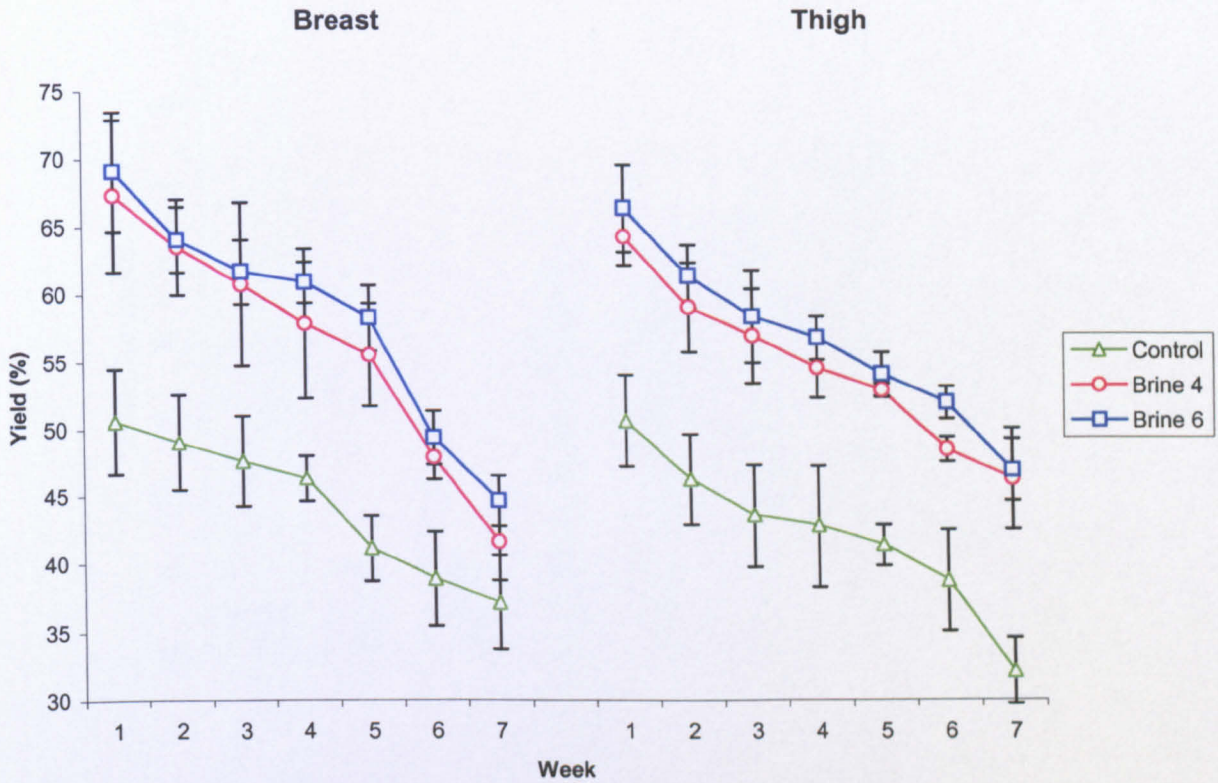
## Oil emulsion model (OEM)

The overall mean yield of the breast was significantly higher than the thigh ( $P < 0.001$ ). Table 23 demonstrates that the overall mean yield of both the breast and the thigh decreased as the age of the birds increased.

**Table 23** OEM overall mean yields (%)  $\pm$  SE of the breast and the thigh affected by age, analysed by general analysis of variance. Within columns (Breast or Thigh), different superscripts denotes significant differences at  $P < 0.001$ .

Age (Weeks)	<u>Breast</u>	<u>Thigh</u>
1	62.3 $\pm$ 1.0 <sup>a</sup>	60.3 $\pm$ 1.2 <sup>a</sup>
2	58.8 $\pm$ 1.2 <sup>b</sup>	55.4 $\pm$ 1.1 <sup>b</sup>
3	56.7 $\pm$ 0.9 <sup>c</sup>	52.8 $\pm$ 0.9 <sup>c</sup>
4	55.0 $\pm$ 0.8 <sup>cd</sup>	51.3 $\pm$ 0.8 <sup>d</sup>
5	51.6 $\pm$ 1.2 <sup>d</sup>	49.3 $\pm$ 1.8 <sup>e</sup>
6	45.3 $\pm$ 1.6 <sup>e</sup>	46.3 $\pm$ 1.3 <sup>f</sup>
7	41.1 $\pm$ 1.3 <sup>f</sup>	41.7 $\pm$ 1.5 <sup>g</sup>

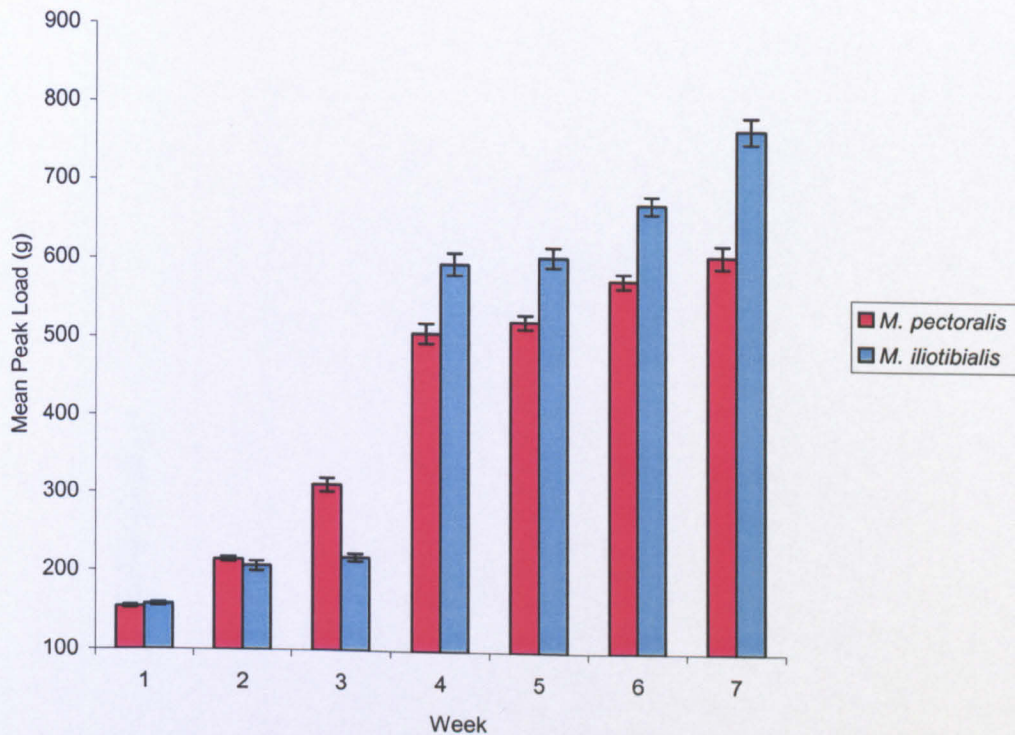
Brine treatments also significantly affected the overall mean yield of the breast and thigh ( $P < 0.001$ ). Figure 27 shows that the brine 6 gave the highest yield, then brine 4 with the controls having the lowest yields. Interaction was seen brine x age, of the birds, with the yield decreasing with an increase in bird age ( $P < 0.02$ ). No other interactions were seen.



**Figure 27** OEM overall mean yield (%) of breast and thigh as affected by age and brine treatment (with standard error bars).

Whole muscle model (WMM) texture

There was a significant difference between the overall mean peak load of the *M. pectoralis* ( $449.1 \text{ g} \pm 5.7$ ) and the *M. iliobtibialis* ( $485.9 \text{ g} \pm 7.3$ ) ( $P < 0.001$ ). The overall mean peak load for the *M. iliobtibialis* was similar to the *M. pectoralis* in weeks 1 and 2 with only a slight increase in week 3. The overall mean peak of the *M. iliobtibialis* was not significantly higher than the *M. pectoralis* until week 4 (Figure 28). There was a significant affect of age on the overall mean peak load. The highest overall mean peak load was at week 7 ( $P < 0.001$ ). There was also a significant interaction between the age and brine treatments (Table 24).



**Figure 28** WMM mean peak load (g) of *M. pectoralis* and *M. iliobtibialis* as affected by age (with standard error bars).

The layer-type *M. pectoralis* did not differ in overall mean peak loads ( $449.5 \text{ g} \pm 7.8$ ) compared to the broiler-type birds *M. pectoralis* ( $448.8 \text{ g} \pm 8.2$ ). This was the same for the *M. iliobtibialis*, with peak loads of  $486.4 \text{ g} \pm 10.5$  and  $485.4 \text{ g} \pm 10.0$  for layer-type and broiler-type respectively. Also, there was no significant affect of diets or sex on the mean peak loads for either the *M. pectoralis* or the *M. iliobtibialis*.

#### Communitied muscle model (CMM) texture

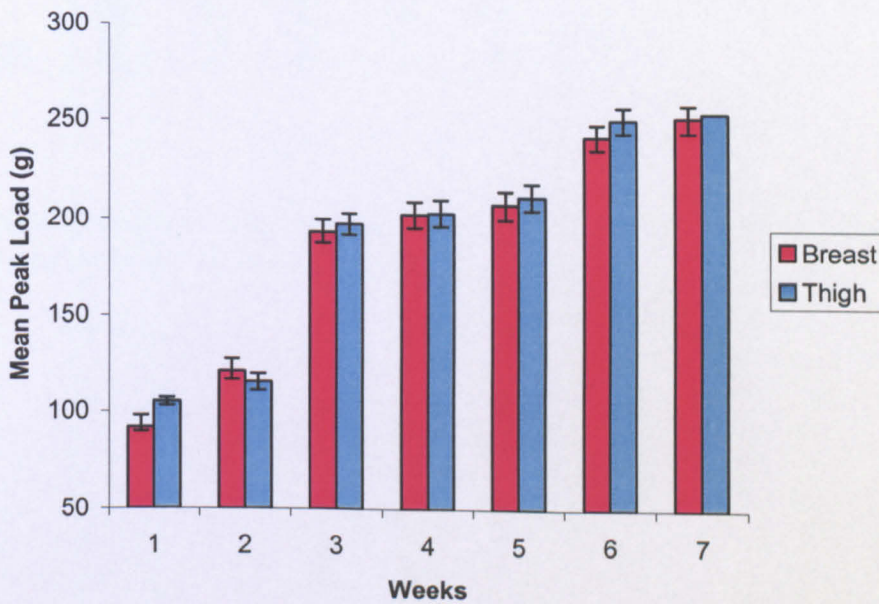
When comparing the breast to the thigh, the thigh gave a significantly higher overall mean peak load of  $196.2 \text{ g} \pm 2.3$  compared to  $190.7 \text{ g} \pm 2.4$  for the breast ( $P < 0.001$ ). Figure 29 shows that with muscle affected by age, the thigh gave consistently higher loads than the breast except on week 2 where the breast gave a mean peak load of  $121.1 \text{ g} \pm 1.1$ , compared to the thigh ( $115.2 \text{ g} \pm 1.3$ ). Generally the peak load for the breast and thigh significantly increased with bird age ( $P < 0.001$ ). Figure 30 displays that both the layer and the broiler-type birds' peak load significantly increased with age and the layer-type birds gave a significantly higher overall peak load of  $194.4 \text{ g} \pm$

**Table 24 WMM overall mean peak loads  $\pm$  SE of the *M. pectoralis* and *M. iliotibialis*, as affected by age and brines, analysed by general analysis of variance. Within rows (Age) and columns (*M. pectoralis* and *M. iliotibialis*) values with different superscripts denotes significant differences at  $P < 0.001$ .**

		<u><i>M. pectoralis</i></u>				<u><i>M. iliotibialis</i></u>			
Age (Wks)	Control (100 % Distilled water)	Brine 1 (4 % NaCl)	Brine 2 (1 % Na <sub>3</sub> P <sub>3</sub> O <sub>10</sub> )	Brine 3 (4 % NaCl and 1 % Na <sub>3</sub> P <sub>3</sub> O <sub>10</sub> )	Control (100 % Distilled water)	Brine 1 (4 % NaCl)	Brine 2 (1 % Na <sub>3</sub> P <sub>3</sub> O <sub>10</sub> )	Brine 3 (4 % NaCl and 1 % Na <sub>3</sub> P <sub>3</sub> O <sub>10</sub> )	
1	194.2 $\pm$ 0.8 <sup>a</sup>	161.1 $\pm$ 0.8 <sup>b</sup>	160.0 $\pm$ 1.1 <sup>b</sup>	104.7 $\pm$ 0.9 <sup>c</sup>	186.5 $\pm$ 1.0 <sup>a</sup>	177.0 $\pm$ 1.2 <sup>b</sup>	150.3 $\pm$ 1.7 <sup>c</sup>	110.6 $\pm$ 0.9 <sup>d</sup>	
2	261.7 $\pm$ 4.2 <sup>a</sup>	225.2 $\pm$ 3.1 <sup>b</sup>	218.9 $\pm$ 3.3 <sup>b</sup>	151.5 $\pm$ 3.6 <sup>c</sup>	295.2 $\pm$ 8.2 <sup>a</sup>	188.7 $\pm$ 1.2 <sup>b</sup>	158.5 $\pm$ 0.8 <sup>c</sup>	118.8 $\pm$ 1.6 <sup>d</sup>	
3	519.7 $\pm$ 9.4 <sup>a</sup>	297.6 $\pm$ 8.4 <sup>b</sup>	242.7 $\pm$ 7.6 <sup>c</sup>	180.1 $\pm$ 4.7 <sup>d</sup>	365.2 $\pm$ 6.7 <sup>a</sup>	231.3 $\pm$ 7.8 <sup>b</sup>	202.9 $\pm$ 12.3 <sup>c</sup>	139.0 $\pm$ 3.3 <sup>d</sup>	
4	795.3 $\pm$ 10.9 <sup>a</sup>	474.2 $\pm$ 9.8 <sup>b</sup>	427.0 $\pm$ 12.0 <sup>c</sup>	290.0 $\pm$ 7.0 <sup>d</sup>	929.6 $\pm$ 11.5 <sup>a</sup>	500.7 $\pm$ 16.2 <sup>b</sup>	461.5 $\pm$ 13.1 <sup>c</sup>	279.7 $\pm$ 8.9 <sup>d</sup>	
5	869.4 $\pm$ 10.9 <sup>a</sup>	458.8 $\pm$ 12.6 <sup>b</sup>	464.6 $\pm$ 10.0 <sup>c</sup>	290.4 $\pm$ 7.4 <sup>d</sup>	976.9 $\pm$ 12.5 <sup>a</sup>	564.7 $\pm$ 11.0 <sup>b</sup>	489.2 $\pm$ 14.7 <sup>c</sup>	308.3 $\pm$ 5.9 <sup>d</sup>	
6	824.7 $\pm$ 12.0 <sup>a</sup>	540.6 $\pm$ 14.4 <sup>b</sup>	514.2 $\pm$ 10.0 <sup>c</sup>	366.4 $\pm$ 13.2 <sup>d</sup>	1113.1 $\pm$ 13.2 <sup>a</sup>	612.4 $\pm$ 10.3 <sup>b</sup>	520.1 $\pm$ 7.3 <sup>c</sup>	396.3 $\pm$ 9.6 <sup>d</sup>	
7	940.5 $\pm$ 15.4 <sup>a</sup>	586.7 $\pm$ 10.9 <sup>b</sup>	600.6 $\pm$ 16.3 <sup>c</sup>	391.0 $\pm$ 16.6 <sup>d</sup>	1169.2 $\pm$ 28.4 <sup>a</sup>	798.8 $\pm$ 13.3 <sup>b</sup>	740.8 $\pm$ 7.3 <sup>c</sup>	415.0 $\pm$ 15.4 <sup>d</sup>	

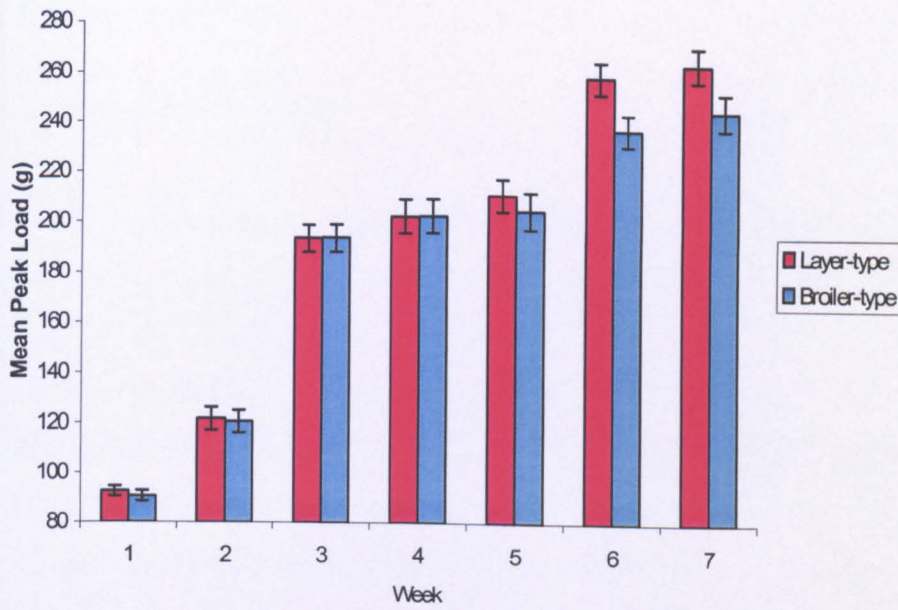
2.4, compared to  $187.2 \text{ g} \pm 2.4$  for the broiler-type ( $P = 0.004$ ). All brine treatments increased the peak loads compared to controls of both the breast and the thigh. Brine 6 gave significantly higher mean peak loads than brine 5, with brine 4 giving the lowest mean peak loads.

There was a significant interaction between brines and age as seen in Figure 31. Regardless of brine treatment all mean peak loads increased with bird age.

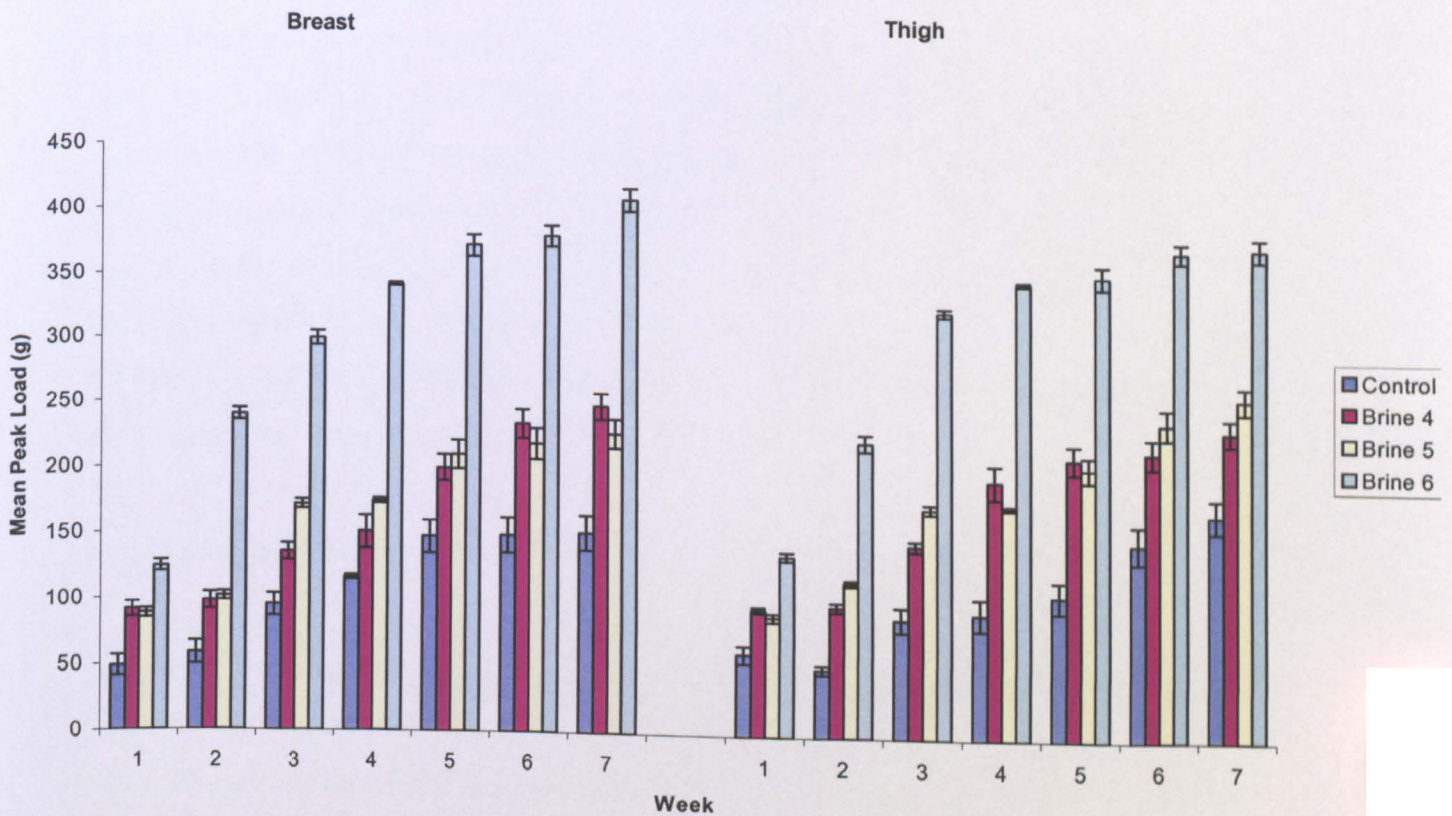


**Figure 29** CMM mean overall peak load (g) of breast and thigh as affected by age (with standard error bars).





**Figure 30** CMM mean overall peak load (g) of broiler-type and layer-type birds as affected by age (with standard error bars).



**Figure 31** CMM overall mean peak load (g) of breast and thigh as affected by age and brine treatment (with standard error bars).

## 6.4 Discussion

### Pre and post-incubation pH

As with experiments 1, 2 and 3, none of the animal production factors affected the pre-incubation pH. A difference may have been anticipated between the broiler-type breast and the layer-type breast due to the difference in the muscle fibre proportions. The spear-probe used for testing pre and post-incubation pH may not have been the best tool, particularly on the smaller muscle samples. The spear-probe was difficult to insert into some of the thigh samples and if the spear-probe was not fully inserted into the muscle this may have resulted in higher pH values.

The post-incubation pH increased compared to that of the pre-incubation pH. The same pattern is seen in experiments 1, 2 and 3. Most swelling was observed with the meat soaked in the presence of NaCl (brines 1 and 4). The swollen appearance of the meat is presumably due to the increase of water retention of the meat. It is reasonable to believe that with the increase in water retention there is also an increase in the chloride ion concentration inside the meat. If all the water originally in the meat blocks (assumed to be 75 % by weight) became equilibrated with the external brine, the concentration of the chlorine in the medium would fall. As the pH of the meat was the same as that of the brine solution there is a strong suggestion that chloride ions have penetrated much or all of the water in the meat.

### Whole muscle model (WMM)

As with experiments 1, 2 and 3, genotype, age, diet and did not have a significant effect on the soaked and cooked yields. Similar results have been expressed in pork meat (Sheard *et al*, 1999) and suggest that the differences between sex, diet and genotype may be not as significant as the addition of NaCl and Na<sub>5</sub>P<sub>3</sub>O<sub>10</sub> either alone or together. Many of the differences between male and female, diets and genotype may have been more evident if the birds where allowed to grow to an older age. This is certainly true with cattle where differences in added water holding between males and

females can only be seen after the cattle are 15 months old (Lawrie, 1998). As the birds age increased, both soaked and cooked yield decreased. This would appear to be a reflection of the amount and conformation of collagen in the meat (Section 1.1.5) and the state of the myofibrillar proteins (Section 1.2.1). The brine treatments significantly increased the soaked and cooked yields of both the *M. pectoralis* and the *M. iliotibialis* in the same manner as in experiment 1, 2 and 3.

#### Comminuted muscle model (CMM)

The yield of the breast and the thigh were significantly affected by the age of the birds but not by any other animal production factor. As with previous experiments (1, 2 and 3) this is partially due to the comminution of structural proteins and partially due to the affect of NaCl and  $\text{Na}_5\text{P}_3\text{O}_{10}$ .

#### Whole muscle model (WMM) texture

In general, increasing age connotated decreasing tenderness. The results here are in broad agreement with Lawrie (1966) and Roman and Ziegler (1974). Ledward (1984) suggested that, as an animal matures, the Schiff base crosslinks decrease in number and are thought to convert to more stable but as yet unidentified structures. These changes contribute to the lower solubility and higher tensile strength of connective tissue in older animals and this may have an effect on meat toughness.

The differences between the breast and the thigh are similar to the work presented by Ouail (1999). Some of these differences can be related to the stromal network (Section 1.1.5). However, as previously described (Section 3.4) great care was taken both with the cooking and the peak load interpretation to remove much of the stromal network influence. Part of difference between *M. pectoralis* and *M. iliotibialis* therefore must be related to the structural proteins, which in turn, may be influenced by the rate and extent of post-mortem decline and the ultimate pH (72 h post mortem). Although the results here indicate that there was not a significant difference between the pre-incubation pH of the *M. pectoralis* and *M. iliotibialis*, the peak load of the *M. iliotibialis* did increase with age more so than the *M. pectoralis*. Although the amount and conformation of collagen in an animal may increase as the animal become older,

and this may increase peak loads, rapid growth of muscle fibres may dilute the relative amounts of collagen in meat so that breast muscles will have a lower peak load than thigh muscles.

Indeed, this may be exacerbated by the fact that within the *M. iliotibialis* the connective tissue content is the major determinant of texture (Wheeler *et al*, 2001). The results here concur, in this experiment the *M. iliotibialis* had a significantly higher soaked and cooked yield compared to the *M. pectoralis*. Thus, it would be anticipated that the higher the cooked yield the lower the texture. This was not the case as the *M. iliotibialis* gave the highest mean peak loads regardless of age and brine treatment.

#### Comminuted muscle model (CMM) texture

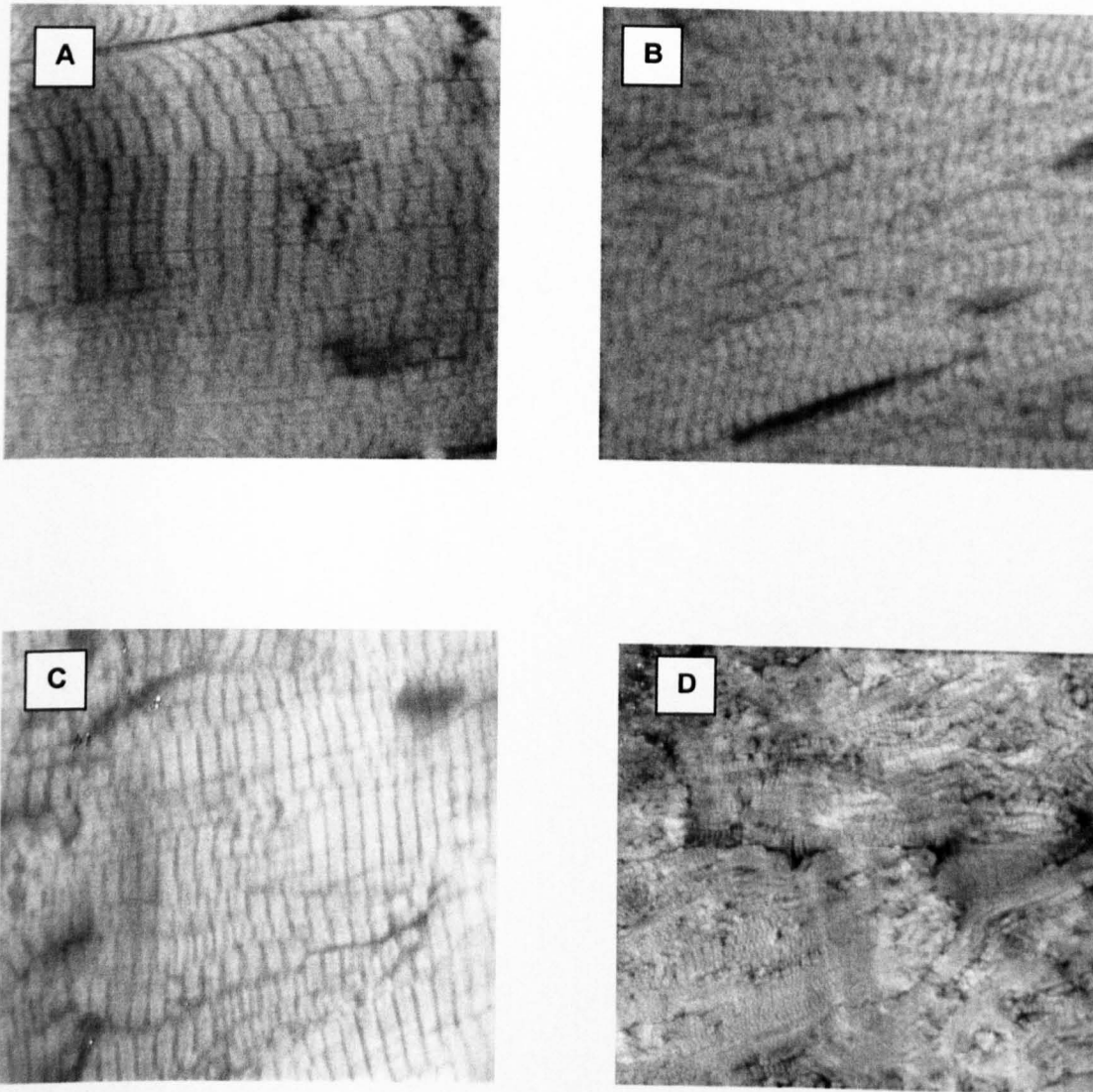
As with the WMM an overall increase in peak load with birds age can be seen. There is also a significant difference between the breast and the thigh with the thigh giving significantly higher overall mean peak loads. Part of the reason for this difference will be related to the amount and conformation of collagen (Section 1.1.5). Berry and Smith (1985), presenting evidence on textural properties of restructured beef steaks, suggested that connective tissue is thought to exert an influence on the texture of restructured meat products similar to its effect on tenderness of intact muscle cuts. Another part of the answer could lie with the diluting effect that rapidly growing muscles (breast) could have compared to slower growing muscles (thigh).

### 6.5 Conclusion

Only age had a significant affect on the soaked, cooked yields and texture of the WMM. The younger birds produced meat which had higher yields and was more tender. Young broiler-type birds produced meat that had the highest yields and the lowest textural results in the CMM. Only the age of the birds significantly affected the OEM with the young birds producing the highest yields.

## 6.6 Microscopy results

Whole muscle model (WMM)



**Figure 32** CLSM (40x) WMM micrographs of breast meat processed in the presence of brine solutions. A = Control, B = Brine 2, C = Brine 3, D = Brine 4.

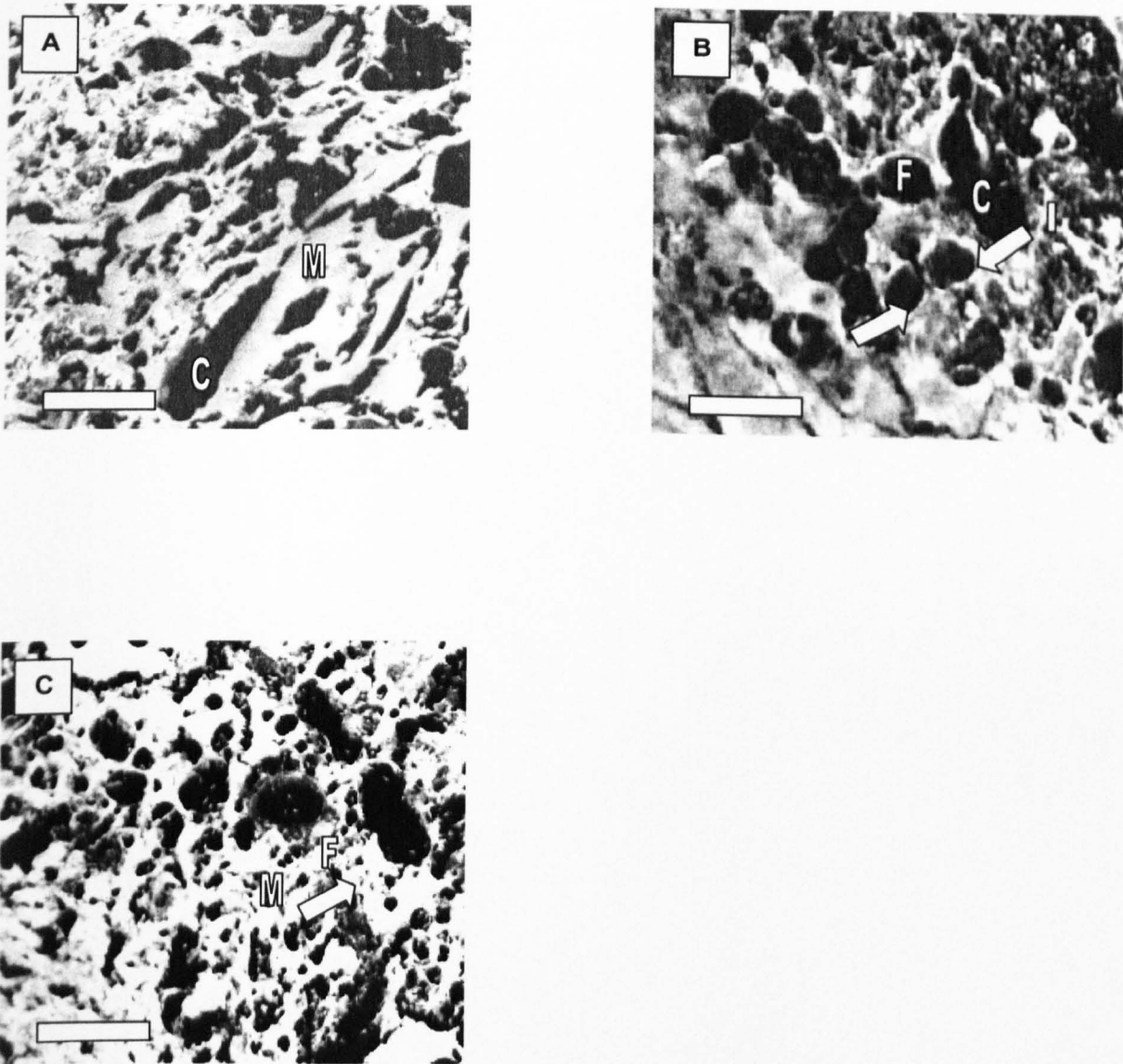
Whole muscle model (WMM)

No differences between animal production factors could be seen within any of the microscopy images. However, differences between brine treatments were evident.

Microscopic images are often difficult to interpret. In the case of WMM, it is difficult to decide which are the remains of M-line and which are the remains of Z-lines, but often this can be resolved as the I-band is generally more dispersed than the H-zone

and in most cases the Z-line is more labile than the M-line. This said, a range of structures could be identified and their interpretation is as follows: (Figure 32, A) Coagulation of proteins with little dispersion other than some loss of thin filaments on cooking. (Figure 32 B) Dispersal of material from H-zone as well as loss of thin filaments; M-line and Z-line generally but not always present. All images from meat subjected to Brine 1 had a cloudy appearance, probably due to the dispersal of sarcoplasmic proteins. (Figure 32 C) Obvious dispersion of H-zone and reduction of M-line; Z-line generally but not always dispersed. All images from meat subjected to Brine 3 had a much brighter appearance compared to those from Brine 1. (Figure 32 D) Extensive dispersion of all regions of sarcomere with only A/I overlap regions visible.

## Oil emulsion model (OEM)



**Figure 33** CLSM (40 x) OEM micrographs of breast meat processed in the presence of brine solutions. A = Control, B = Brine 4, C = Brine 6, Bar = 100  $\mu\text{m}$ , C = channels, F = intact fat cells (arrows indicate), M = matrix, I = interfacial protein film (arrow indicates).

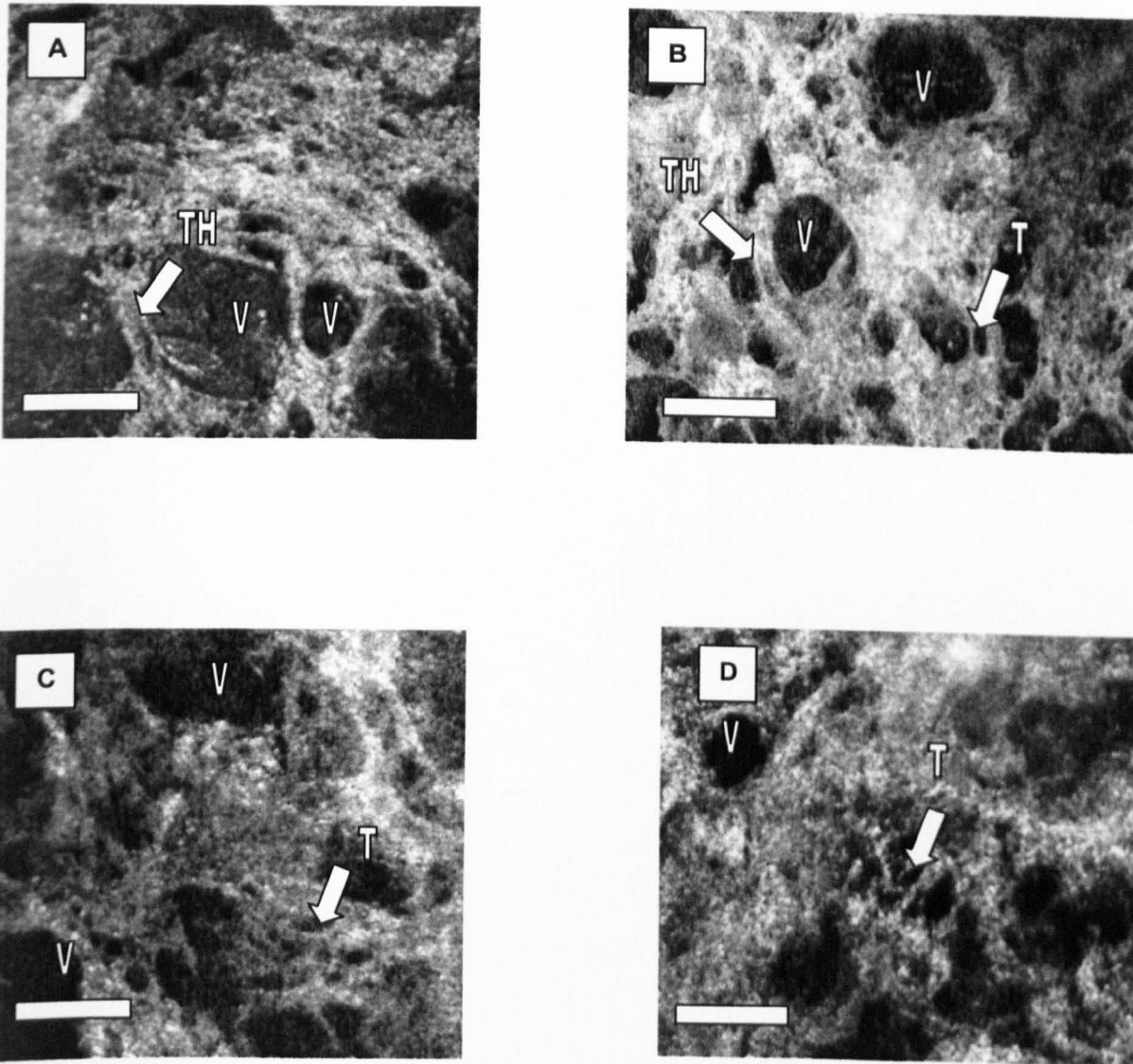
## Oil emulsion model (OEM)

No differences between animal production factors could be seen within any of the microscopy images. However, differences between brine treatments were evident. Generally the emulsions had a fairly even distribution of fat throughout the matrix, with fat globules generally assuming irregular shapes. Many of the fat globules were surrounded by a defined dark line which is believed to be the interfacial protein film

encapsulating fat globules (Hansen, 1960). CLSM revealed that the matrix was highly aggregated, with large, well linked tunnels which were often filled with fat from unstable globules which lacked an intact interfacial film. The brine control (Figure 32 A) produced an emulsion that had large fat channels and a discontinuity of the protein matrix. This coalescence of fat was strikingly evident in all the brine controls and in some cases occupied about 50 % of the area, however, a few very small, round globules could also be seen. This may have led to the flaccid nature of the meat emulsions (Section 2.6) which prevented them from being used for texture analysis. Brine treatment 4 (Figure 32 B) produced an emulsion which had a higher density of the protein matrix which occupied a large part of the area. This brine treatment also had a higher yield (Section 6.3) compared to the controls. Within the protein matrix there were some irregularly shaped fat particles (arrows indicates) that seemed to be in the intermediate stage of coalescence. Emulsions made with brine 6, show fairly even fat distribution with a large protein matrix. There was a decreased density of fat particles that did not coalesce, some of the larger globules were irregular in shape probably resulting from some structural constraint imposed by the protein matrix.



## Comminuted muscle model (CMM)



**Figure 34** CLSM (40x) CMM micrographs of breast meat processed in the presence of brine solutions A = Control, B = Brine 4, C = Brine 5, D = Brine 6, Bar = 100  $\mu\text{m}$ , T = thread-like structures (arrows indicate), TH = thick protein strands (arrows indicate), V = voids.

## Comminuted muscle model (CMM)

No differences between animal production factors could be seen within any of the microscopy images. However, differences between brine treatments were evident.

The matrices of all the emulsions had basically a similar appearance. The three dimensional images exhibited two types of structure: globules and small aggregates connected by long coiled filaments (Figure 34). The microstructure of the emulsions showed some distinctive differences that tended to correspond to differences in texture (Section 6.3).

The brine controls produced a protein matrix that had a fine, thread-like structure, which was highly interwoven. The thread-like structure had randomly oriented with large irregular open spaces within it. Some areas had an irregular, loose, lacy network with smaller voids. Some larger voids appeared to produce channels; some of these channels seemed to be interconnected. It could be imagined that moisture is channeled away from the centre of the meat emulsion leading to decreased cooked yields (Table 20 - 22 and Figure 26).

Brine 4 produced emulsions with some aggregates which were linearly oriented to form thick strands which were connected by fine strands. These formed an ordered, open continuous matrix of linear thread-like strands. The strands varied in size, although generally were larger and thicker than those observed in brine controls. Individual structures within large clusters were difficult to identify, but some parallel filaments were observed.

Brine 5 produced a structure that had a disordered, more compact, locally dense structure which corresponded to the aggregated clusters viewed in brine 4. A continuous lacy network with small voids was also observed. The continuous network contained strands which appeared to be composed of cross-linked filaments. Fine filaments connected the strands. The fine filaments were thicker than those in brine 4.

The emulsions produced by Brine 6 contained a regular lattice network of filaments and small voids interrupted by irregular, dense regions of protein. The dense regions were much more solid than those observed in brines 4 and 5. Some emulsions (Figure 34 D) produced a cohesive, well-structured matrix with a highly interconnected network of strands in which a discontinuous protein matrix with indistinct features could be seen.

### 6.6.1.1 Microstructure related to texture

#### Whole muscle model (WMM)

Relationships between the microstructure and texture can only be speculative. However, it seems reasonable to assume that as the dispersion of the meat proteins increases there was a general loss of structural integrity of the muscle. This dispersion of meat proteins, principally myosin, was related to the brine treatments. Meat with little dispersion (Figure 32 A, controls) produced meat with significantly higher mean peak loads compared to Figure 32 B and Figure 32 C (brines 1 and 2 respectively). Most dispersion appeared in Figure D, brine 3 which in turn produced meat which had the lowest mean peak loads. Importantly, brine 3, produced meat which had the highest soaked and cooked yields (Section 6.3). Undoubtedly, part of the reason why these meats had the lowest mean peak loads was due to the increase in added water between meat structures.

#### Comminuted muscle model (CMM)

Textural differences were possibly caused by what appears to be differences in void size and porosity as well as degrees of inter-linking with other protein matrices and water loss during cooking. Generally brine 6 resulted in the highest peak load (Section 6.3), as can be seen from Figure 34 D these emulsions have relatively small voids and a highly interwoven protein matrix. Brine 6 also resulted in the highest overall mean cooked yield (Section 6.3). Brine 5 (Figure 34 C) produced emulsions that had larger voids and a less dense protein matrix producing an almost sponge-like appearance and suggesting a lower integrity in the protein matrices. The overall mean peak load for brine 5 was significantly lower than that of brine 6 (Section 6.3). Montejano *et al* (1984) found that beef muscle emulsions with a sponge-like texture and large pores had low elasticity. They also indicated that these emulsions had lower shear stress (shear stress has been correlated to hardness). The texture results (Section 6.3) reflect this lack of inter-linking and increase void size. Furthermore, brine 5 produced a lower cooked yield, which could be interpreted as the emulsions inability to 'hold' moisture within its matrices. The results of this study support the findings of Gordon and Barbut (1989) and Gordon and Barbut (1990), these studies site scanning electron

and transmission electron micrographs, which, although not directly comparable, some similarities can be drawn. Particularly in relation to the channels described above, Gordon and Barbut (1990) describe these channels as tunnels and indicate that they are formed prior to cooking. During cooking moisture is lost through them. These channels could also lead to the reduced protein matrix integrity weakening the emulsion.

### Oil emulsion model (OEM)

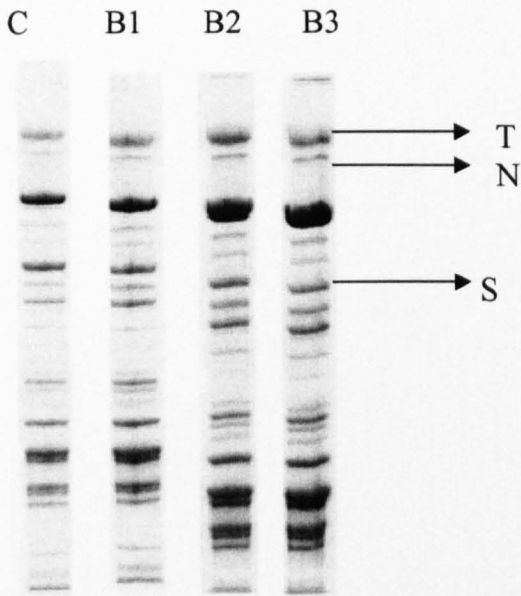
Hermansson (1986) reported that all fat in emulsions was surrounded by some kind of protein film regardless of whether it was in the form of intact fat cells, dispersed droplet or pools of fat. This appears to be true in brines 6 and 4 (Figure 33 B and C) but not for control (Figure 33 A). The latter had a significantly reduced yield (Section 6.3). This reduced yield probably occurred because of poor fat stabilization, resulting from inadequate interfacial protein film (IPF) formation in combination with the formation of a weak, incoherent matrix, which filled with large discontinuities (channels). These channels are probably formed before the mix is cooked, forming a route out for the unstable fat. The large pores and weak nature of the matrix cannot adequately physically entrap the fat in a similar fashion to the CMM. On cooking, further aggregation and shrinkage takes place and the molten fat is therefore able to leave more easily. When thermal energy is introduced into the comminuted mix during cooking, it probably enhances protein to protein interactions and increases the effectiveness of the chloride ions as a binding agent. Since the proteins are closer together in the OEM than in any other model, aggregation takes place which includes the proteins involved in fat binding. This could be the result of a change in the thermodynamics of the system to favour protein-to-protein interactions over protein-to-lipid interactions, thereby causing widespread IPF disruption. The increased protein-to-protein interactions cause the channels which were present to become much wider, and unstable molten fat would be free to leave the mix. The destabilised emulsions lost not only fat, but also moisture (Section 2.5.3), indicating that a non-uniform dispersion of fat could cause a disruption of the water binding of protein in a given comminuted meat system. The moisture dependent fat dispersion can be explained by an increased ability of the matrix to restrict fat mobility with an increase in the moisture dependent viscosity of the protein matrix. Comminution of chicken

meat with oil in the presence of NaCl alone (brine 4) and NaCl and  $\text{Na}_5\text{P}_3\text{O}_{10}$  (brine 6) formed emulsions that led to an increased yield after cooking. Several studies have shown that salt-soluble proteins are involved in fat binding and for IPF around fat particles (Section 1.3.3). It is therefore likely that the Na ion plays an important role in the formation of the IPF around fat particles. The synergistic effect of NaCl and  $\text{Na}_5\text{P}_3\text{O}_{10}$  could be due to the action of salt-insoluble proteins. The increase in the pH (Section 6.3) seen with the addition of  $\text{Na}_5\text{P}_3\text{O}_{10}$  could release these salt-insoluble proteins. The salt-insoluble proteins could complement the IPF of the salt-soluble proteins forming a very dense matrix around fat particles and increasing the fat binding.

### Conclusion

Brine treatment and comminution significantly affected the meat structure. With an increase in protein dispersion there is an increase in soaked and cooked yield and a decrease in mean peak yield (WMM). With an increase in interwoven protein fibres and a decrease in porosity and void size there is an increase in cooked yield and “bind” (CMM). A combination of emulsification and physical restriction of fat coalescence provide for a stable emulsion (OEM).

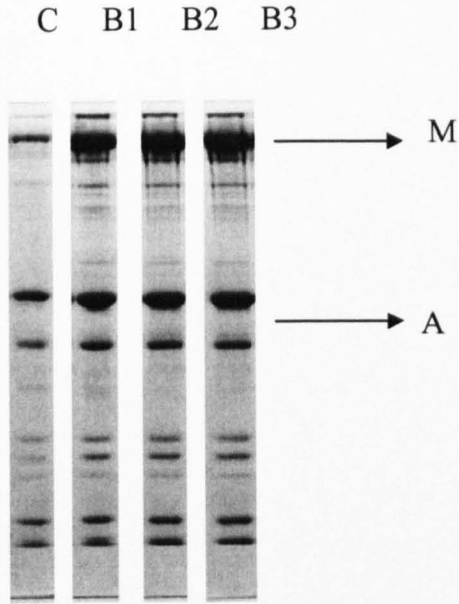
## 6.7 Electrophoresis



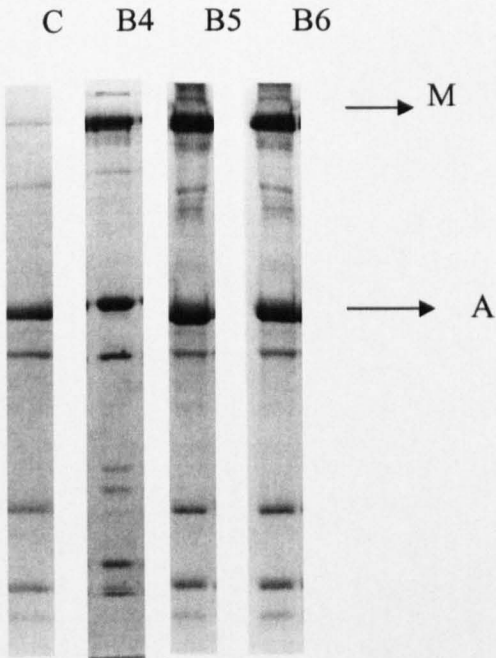
**Figure 35** SDS-Page separation of raw breast processed in the presence of brine solutions C= control; B1= Brine 1; B2 = Brine 2; B3 = Brine3, T = titin, N = nebulin, S = standard ( $\beta$ -galactosidase).

Figure 35 demonstrates that the addition of NaCl (brine 1) and  $\text{Na}_5\text{P}_3\text{O}_{10}$  (brine 2) alone or in combination (brine 3) did not split titin and nebulin (Section 1.1.4) into their relative components. The meat samples that had been subjected to brines 3, 4 and 5 produced polyacrylamide gels that were unusable. These gels tended to look washed out and were associated with worsening sharpness of protein bands.

Figure 36 demonstrate that the addition of NaCl and  $\text{Na}_5\text{P}_3\text{O}_{10}$  split the myosin and actin bands. Lanes B1 and B2 have darker lines underneath the main myosin and actin bands suggesting that the addition of NaCl and  $\text{Na}_5\text{P}_3\text{O}_{10}$  is splitting the protein chains. Furthermore, lane B3 has distinct stained lines underneath the main line, suggesting that the combined use of NaCl and  $\text{Na}_5\text{P}_3\text{O}_{10}$  split the proteins, particularly myosin, even further than NaCl and  $\text{Na}_5\text{P}_3\text{O}_{10}$  alone.



**Figure 36** SDS-Page separation of raw breast processed in the prescence of brine solutions. C= control; B1= Brine 1; B2 = Brine 2; B3 = Brine3, M = myosin, A = actin.



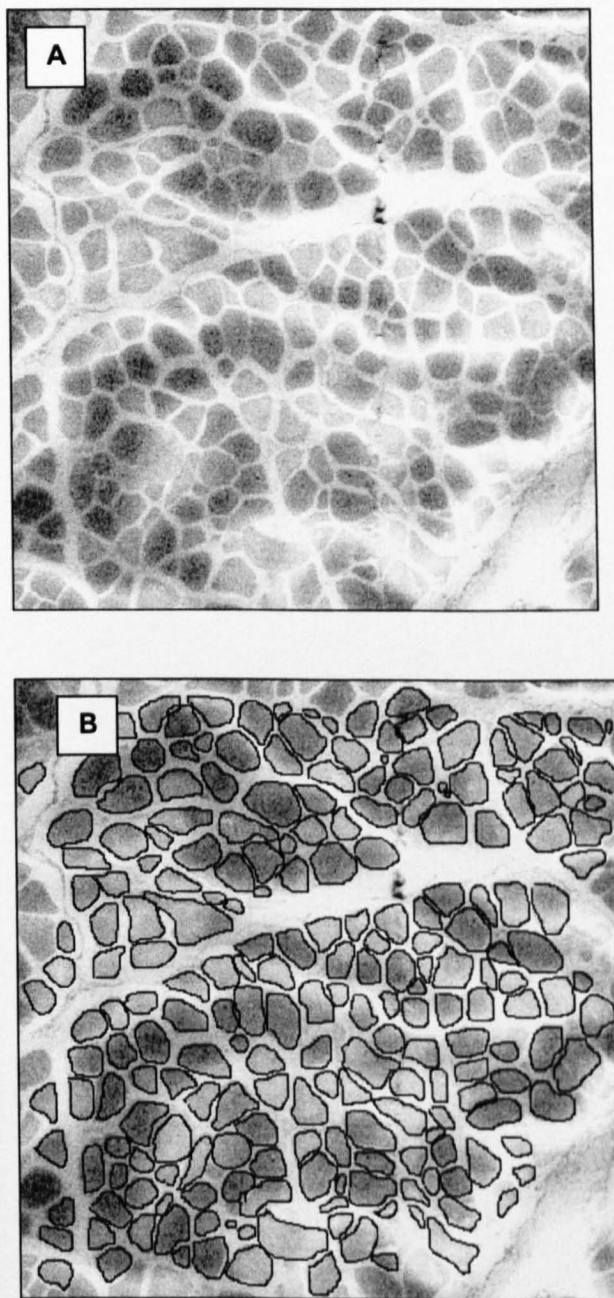
**Figure 37** SDS-Page separation of raw breast processed in the prescence of brine solutions. C= control; B4= Brine 4; B5 = Brine 5; B6 = Brine6, M = myosin, A= actin.

The results of the SDS page gels support the results discussed in Section 3.4. The controls for all gels show a decrease in the pH which facilitates the dispersal of the sarcoplamic proteins (Section 1.1.4). In the sarcomere, this dispersal of sarcoplamic proteins could facilitate the action of NaCl on the functional proteins actin and myosin (see Section 1.3.1; Figure 35 B1; Figure 36 B1; Figure 37 B4).

It is likely that the main actions of NaCl are the dispersion of myofibrillar proteins; probably the A/I overlap at regions rich in actomyosin. The action of phosphate can also be attributed to an increase in ionic strength and a pH shift away from the isoelectric point in meat products (Schmidt and Trout, 1982; Lewis *et al*, 1986; Trout and Schmidt, 1986). However, when NaCl is combined with  $\text{Na}_5\text{P}_3\text{O}_{10}$ , there is a synergistic action (Section 1.3.2). This synergistic action is probably as result of the increasing pH and ionic strength of the brine solution. This increase in pH and ionic strength would facilitate the precipitation of the sarcoplamic proteins from the sarcoplasm, NaCl could then disperse that myofibrillar proteins. On heating, the dispersed myofibrillar proteins form a gel which retains water within the meat structure. Improved water holding capacity through the formation of myofibrillar gels is supported by many others (Velinov *et al*, 1990; Wang and Smith, 1992; Nielsen *et al*, 1995; Moiseev and Cornforth, 1997; Nowsad *et al*, 2000; Mielnik *et al*, 2002; Tsai-Fuh Tseng *et al*, 2002) and not only in poultry meat, but also in pigs (Sheard *et al*, 1999; Fernández-Martín *et al*, 2002), and in beef (Boles and Swan, 2002). The formation of myofibrillar gels also increases the binding capacity of the comminuted meat (Siegel and Schmidt, 1979a; Nielsen *et al*, 1995; Nowsad *et al*, 2000; Mielnik *et al*, 2002).

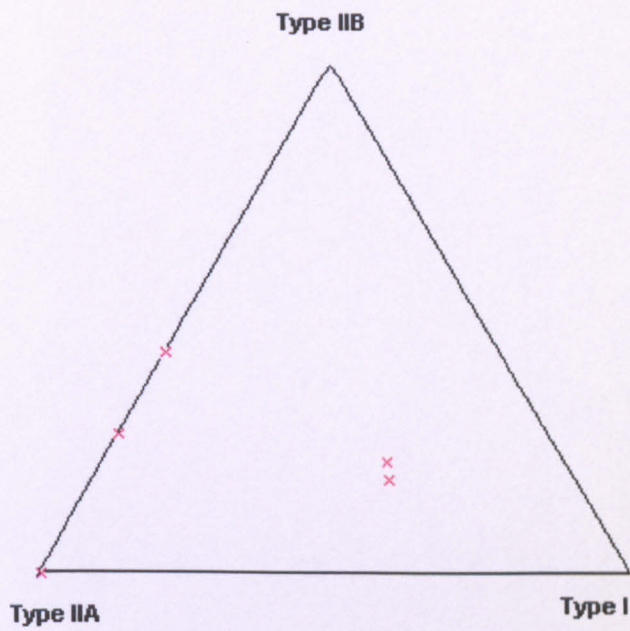


## 6.8 Muscle fibre typing

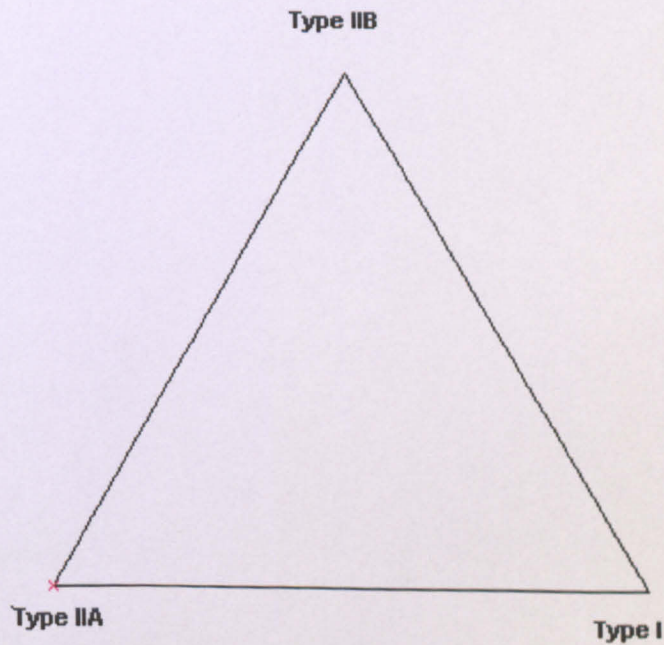


**Figure 38** A) Histological demonstration of muscle fibre types with ATPase. B) Contour lines for the semi-automated segmentation for muscle fibre type measurement.

*M. pectoralis*

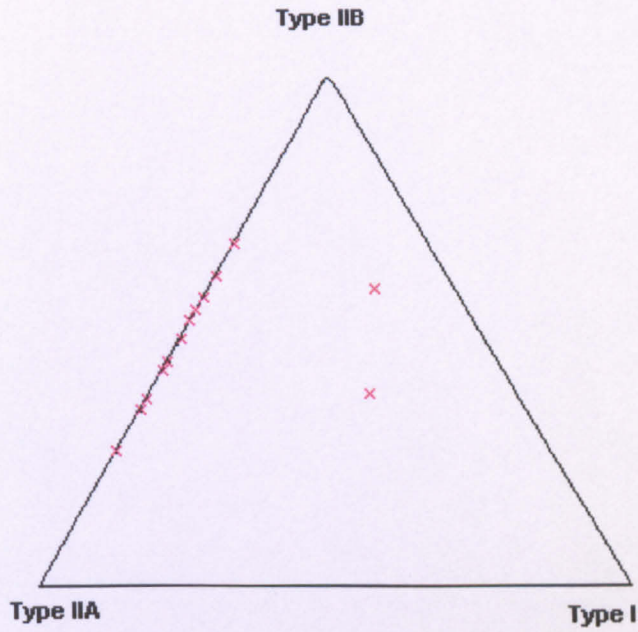


*M. iliotibialis*

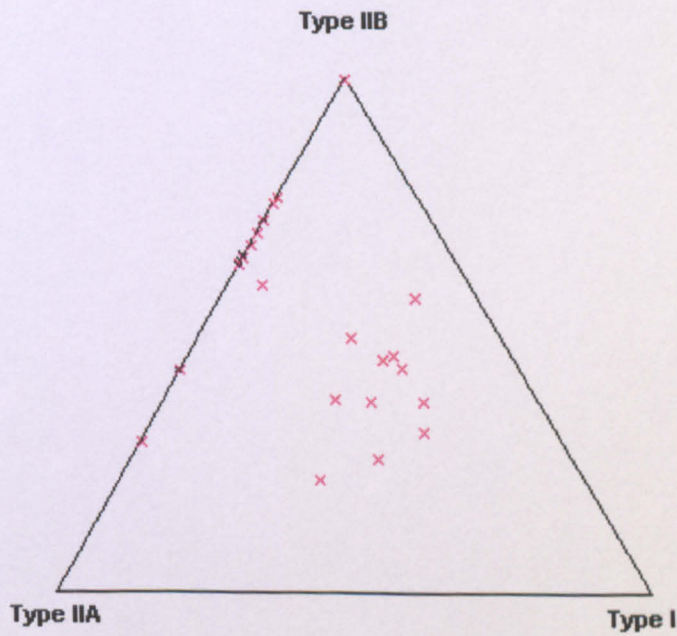


**Figure 39** Ternary diagram of Week 1 muscle fibre proportions of *M. pectoralis* and *M. iliotibialis*. Red crosses indicate the distribution of all muscle fibre types towards either type I, type IIA or type IIB.

*M. pectoralis*



*M. iliotibialis*

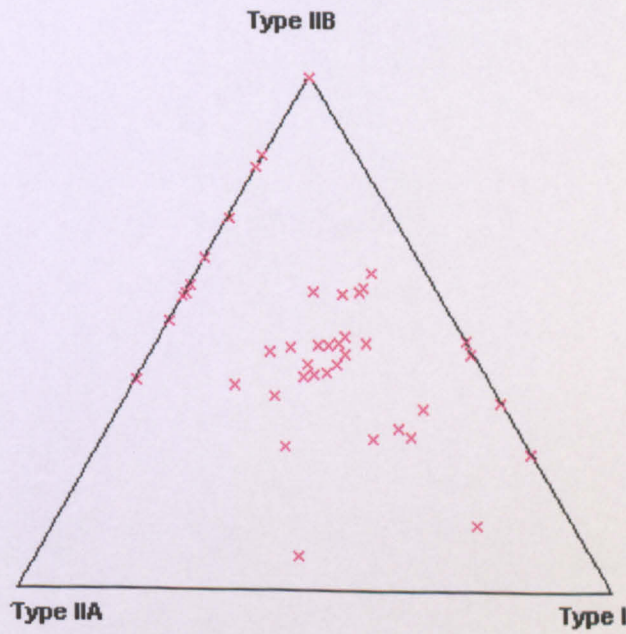


**Figure 40** Ternary diagram of Week 2 muscle fibre proportions of *M. pectoralis* and *M. iliotibialis*. Red crosses indicate the distribution of all muscle fibre types towards either type I, type IIA or type IIB.

*M. pectoralis*

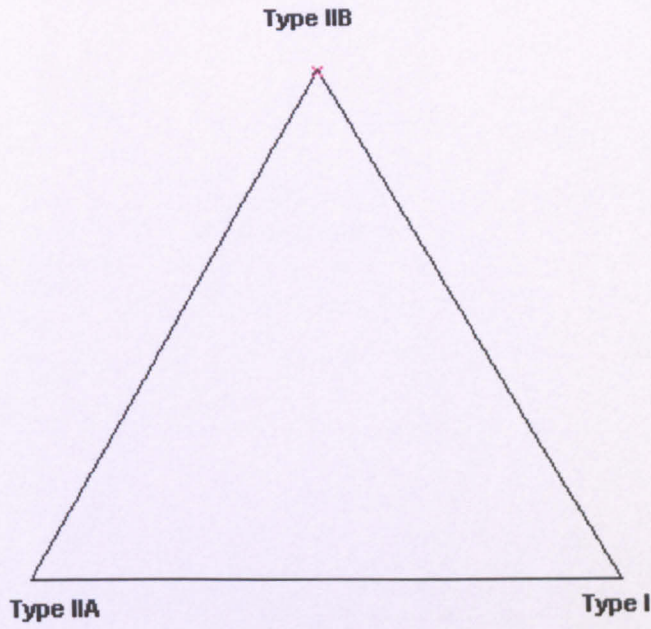


*M. iliotibialis*

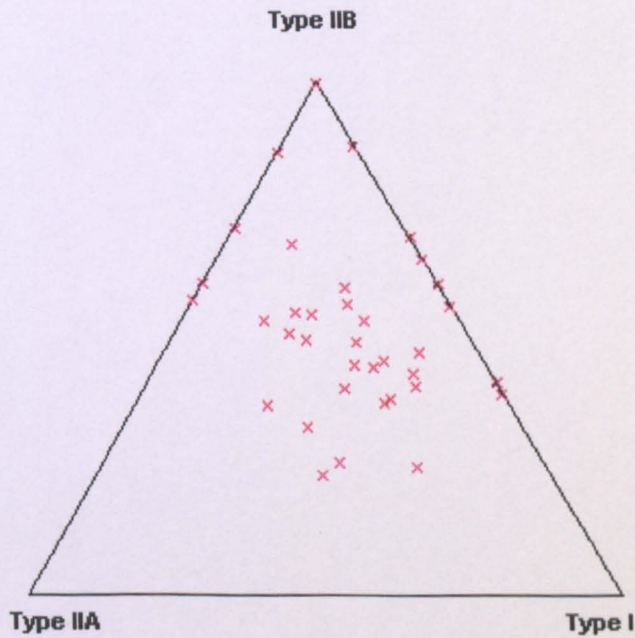


**Figure 41** Ternary diagram of Week 3 muscle fibre proportions of *M. pectoralis* and *M. iliotibialis*. Red crosses indicate the distribution of all muscle fibre types towards either type I, type IIA or type IIB.

*M. pectoralis*

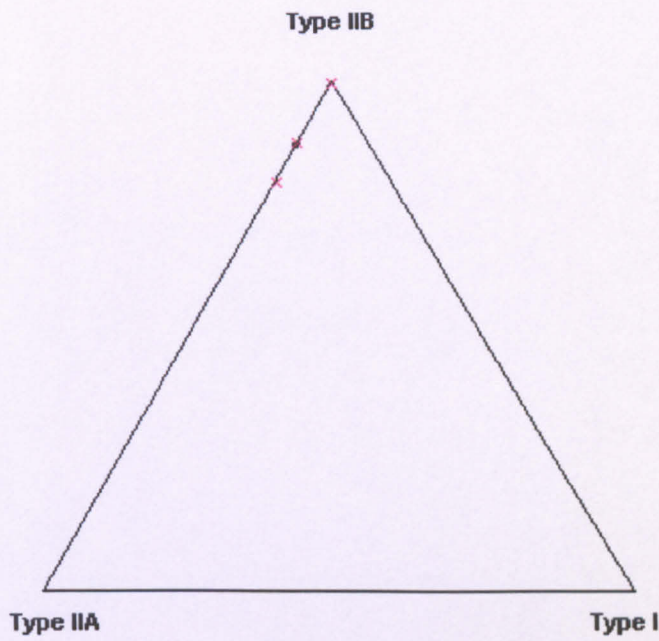


*M. iliotibialis*

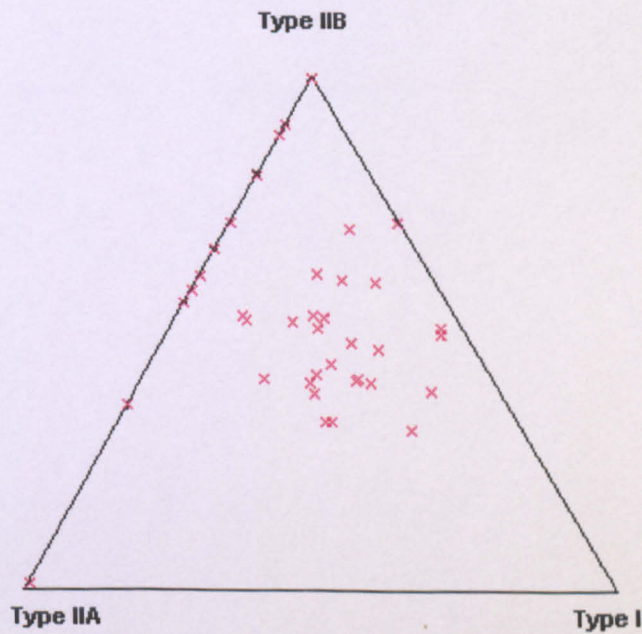


**Figure 42** Ternary diagram of Week 4 muscle fibre proportions of *M. pectoralis* and *M. iliotibialis*. Red crosses indicate the distribution of all muscle fibre types towards either type I, type IIA or type IIB.

*M. pectoralis*

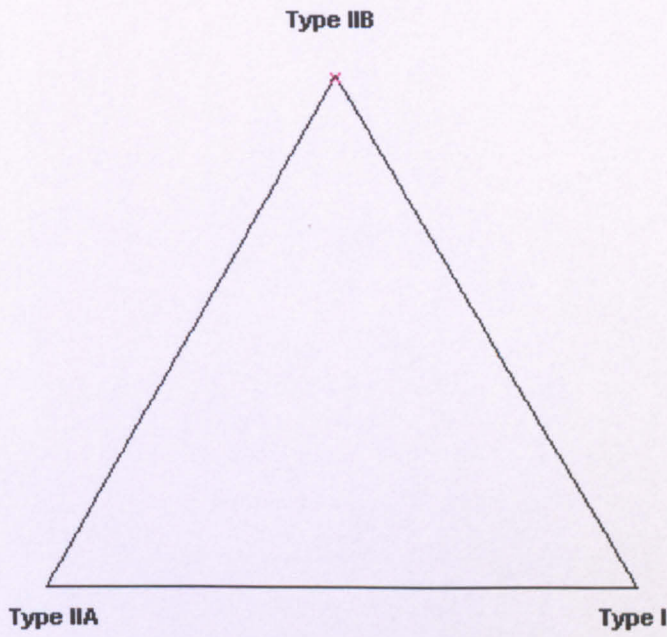


*M. iliotibialis*

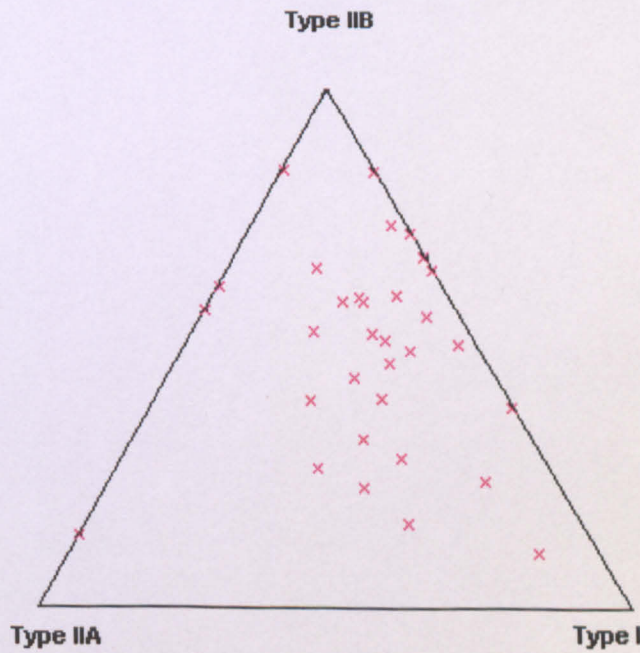


**Figure 43** Ternary diagram of Week 5 muscle fibre proportions of *M. pectoralis* and *M. iliotibialis*. Red crosses indicate the distribution of all muscle fibre types towards either type I, type IIA or type IIB.

*M. pectoralis*

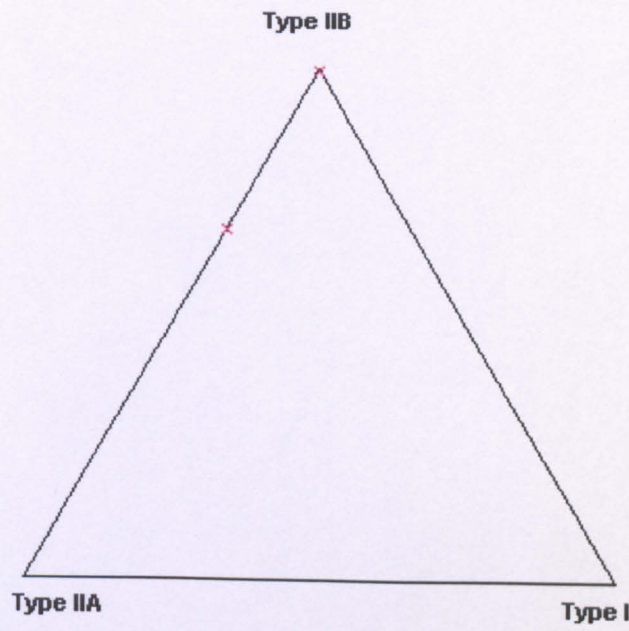


*M. iliotibialis*

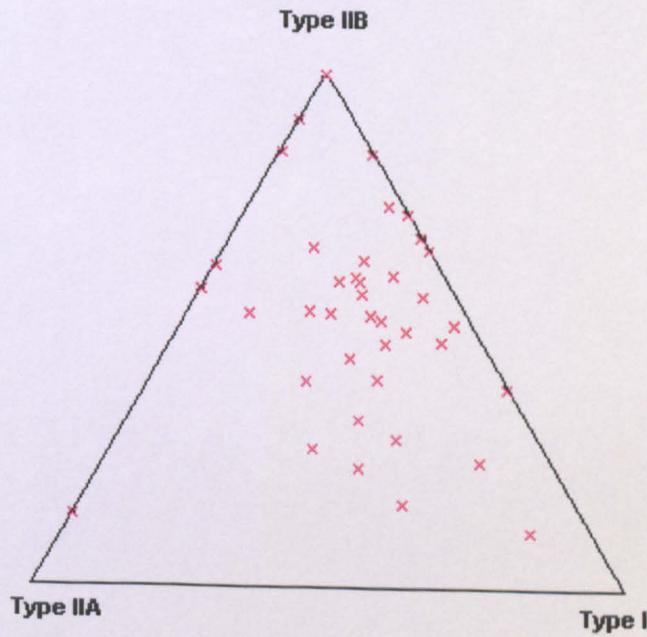


**Figure 44** Ternary diagram of Week 6 muscle fibre proportions of *M. pectoralis* and *M. iliotibialis*. Red crosses indicate the distribution of all muscle fibre types towards either type I, type IIA or type IIB.

*M. pectoralis*



*M. iliobtibialis*



**Figure 45** Ternary diagram of Week 7 muscle fibre proportions of *M. pectoralis* and *M. iliobtibialis*. Red crosses indicate the distribution of all muscle fibre types towards either type I, type IIA or type IIB.



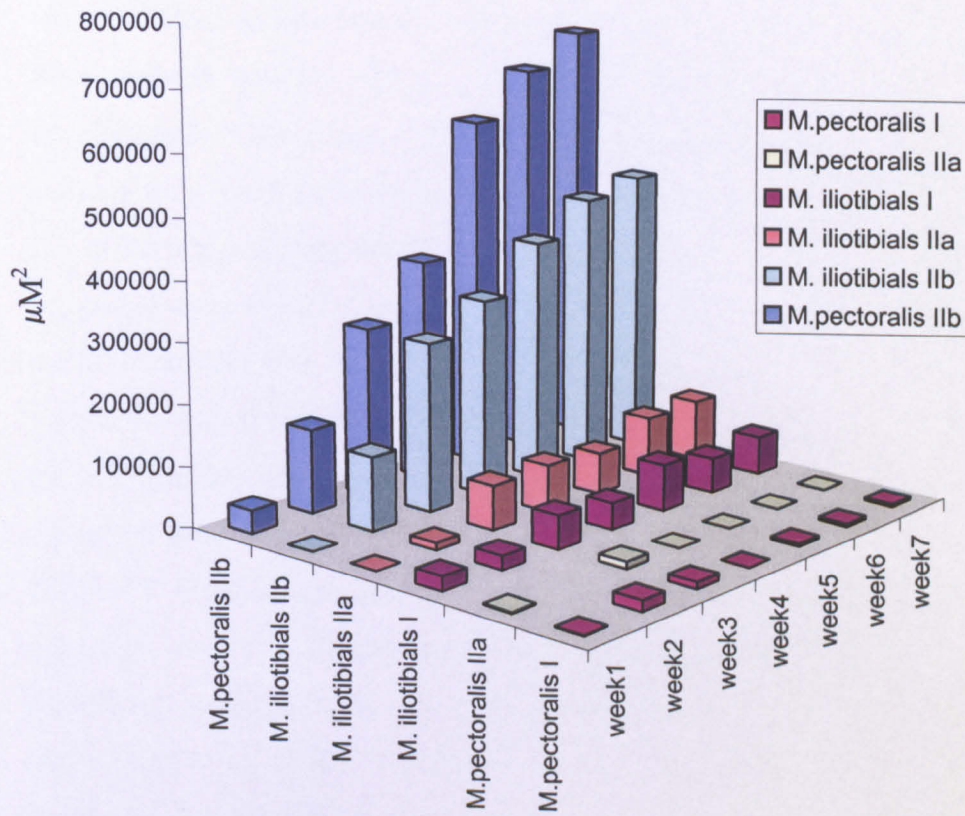


Figure 46 Muscle fibre size as affected by muscle type and age.

## 6.9 Discussion

### Muscle fibre proportion

These results (Figures 39-45) suggest that with animal age muscle fibre proportions change. These results broadly agree with work of Ashmore and Doerr (1971a) who have showed that IIA fibres transform to larger diameter IIB fibres during the development of the chick, and it has been suggested that selection for muscle size in animals may result in an accumulation of factors that promote greater conversion of IIA to IIB fibres during development (Ashmore *et al*, 1972). Certainly the results here suggest that the broiler-type birds have a larger proportion of type IIB muscle fibres in the *M. pectoralis* than the layer-type. However the muscle fibre composition of the *M. iliotibialis* was more diffuse. The difference in fibre type composition could be caused by a functional differentiation in muscle. Muscles involved in posture are more oxidative than those involved in movements (Totland and Kryvi, 1991; Henckel, 1995). In pigs, it has been demonstrated that the deepest muscles of the limbs generally have the highest percentage of slow oxidative type I fibres, while the superficial muscles have the highest percentage of glycolytic (Type IIB) fibres (Armstrong *et al*, 1987). Type II myofibres in the chicken have a faster contraction speed and more capability for anaerobic metabolism than type I myofibres (Ashmore and Doerr, 1971b). Ashmore *et al* (1972) suggested that domestication of animals and selection for muscularity promotes concentration that favour conversion of type II red to type II white myofibres.

### Muscle fibre size

The results here support the evidence that there is an inverse relationship between fibre diameter and the oxidative capacity of muscle fibres. Type I are the smallest, type IIB fibres have the largest diameter, and IIA have an intermediate size (Cassens and Cooper, 1971; Rosser *et al*, 1992).

The difference between the broiler and layer-type birds muscle size is comparable to the work of Aberle and Stewart (1983). They concluded that the broiler-type birds yield a higher percentage of meat and lower percentage of bone compared to layer birds. Smith (1963) reported that broiler type birds had larger diameter muscle fibres

than layer birds at the same age. Mizuno and Hikami (1971) also found greater numbers of myofibres in broiler than layer birds. Smith (1963) presented indirect evidence that the broiler line had more muscle fibres than the layer birds, but concluded that fibre size was more important than fibre number in determining muscle size in these lines.

Ashmore and Doerr (1971a) have also shown that IIA fibres transform to larger diameter IIB fibres during the development of the chick, and it has been suggested that selection for muscle size in animals may result in an accumulation of factors that promote greater conversion of IIA to IIB fibres during development (Ashmore *et al*, 1972). The results here support Ashmore and Doerr (1971a) and Ashmore *et al* (1972). Considering these results, it does seem probable that the *M. pectoralis* muscle of chickens without the ability for long term flight has only very few type I fibres and is a contrast to that of the *M. iliotibialis* which is a postural muscle containing a larger proportion of type I fibres. These fibres are thought to play an important role in long-term locomotion rather than the IIB fibres. Type IIB fibres are believed to be adapted for only short-term intense activity. The main difference in muscle fibre type composition seems to be caused by a functional differentiation in muscle. As with previous results (experiments 1, 2, 3 and 4) the *M. pectoralis* is the more sensitive of the muscles in as far as affects of genotype, age, sex and diet.

## Chapter 7

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### General Discussion

The aims of this project were to describe the relationships between animal production parameters and meat processing functionality, plus the underpinning science of protein functionality under different processing situations. The functional properties of meat seem to be determined less by animal production parameters and more by processing factors.

The pre-incubation pH of the meat across all the studies ranged from 5.6 (experiment 1) to 5.9 (experiment 4) in the *M. pectoralis* and from 5.6 (experiment 2) to 6.1 (experiment 4) in the *M. iliobialis*. The pre-incubation pH was little affected by any animal production factors, and consequently this had little effect on the processing functionality of the meat. Given that most meat was subsequently soaked in a brine solution, a lot of the possible effects of the pre-incubation pH may have been reduced. As significant differences were seen in the meat soaked in the control brines. The broiler-type birds pH was significantly higher than in the layer-type birds.

It would be anticipated that significant differences would be seen between the broiler and the layer-type birds across all experiments. Significant differences were seen in experiment 3 (WWM) (Section 3.3) with broiler-type birds producing higher soaked and cooked yields. The broiler-type birds gave softer products with lower mean peak loads than the layer-type in the WMM with experiments 1, (Section 3.3) 2 (Section 4.3) and 3 (Section 5.3). One possible reason for the few significant differences between the broiler and the layer-type birds may be that the broiler-type birds were the Ross 308 strain, which is bred to be versatile to satisfy a broad range of end-product requirements. The JA 57 is a semi-industrial layer strain which is commonly used for the slow-growing bird niche markets used for eggs and meat. The JA 57 females can be mated to standard industrial broiler-breeder males, which reach slaughter weight in 42 days, to produce a semi-industrial broiler, which reaches slaughter weight in 56 days. Thus, although referred to as the layer-type within this thesis, the JA 57 does have some traits that lend themselves to the broiler market. These broiler-type traits may have reduced some of the significances anticipated from

some of the experiments particularly pre-incubation pH. The slaughter age of the birds was determined partly to assess the effect of genotype on meat processing characteristics, because different strains of birds have to be compared at the same age and partly because other parts of the birds were used in other trials. The difference between the broiler-type and layer-type birds may have been further emphasized had the birds been allowed to grow older.

Experiments 2 and 4 confirmed that age has a significant effect on the functional properties of meat. With the exception of the OEM and WMM texture (experiment 2) (Section 4.3), all functionality models showed a negative effect on binding properties with an increase in bird age. The results shown here are comparable with Bendall and Restall (1983), Wang and Smith (1992) and Swatland and Barbut (1999b), with regards to the added water holding capacity of the meat; Voller *et al* (1996), Nowsad *et al* (2000) and Qiao *et al* (2001) with regards the binding properties; with Voller *et al* (1996) with regards the microstructure of meat emulsions; and with Breidenstein (1982); Berry (1987); Liu *et al* (1996) and Voller *et al* (1996) with regards the mechanical texture of the meat.

There were no significant effects of sex on any of the functional models. There was, however, a significant interaction between sex x genotype and genotype x sex x brines x diets was seen in experiment 3 OEM (Table 14), WMM texture (Section 4.3) respectively. Generally the male broiler-type fed a high protein diet and subsequently soaked in brine 3 (4 % NaCl and 1 %  $\text{Na}_5\text{P}_3\text{O}_{10}$ ) produced the meat which had a greater soaked and cooked yield and was more tender than other meats. The result for the OEM seems unlikely as this model was the most comminuted and therefore less likely to be affected by the animal production factors. One of the problems with the OEM is the balance between water, oil and meat and comminution. Too much of either additive or comminution results in a emulsion sample (model) in which the oil droplets separate into a concentrated layer on the top of the emulsion sample or the oil droplets form small or large flocs, with no change in the droplet size, within the emulsions model. Alternatively a coalescence of the oil occurs forming large spherical droplets, which break the emulsion model partly into two. The OEM chosen for this thesis was a delicate balance between an emulsion sample that would provide useful information for the thesis and model that was similar to that used in

meat industry. If an alternative emulsion sample was chosen then possibly some of the animal production factors may have been seen.

Experiments 3 and 4 demonstrated that diet had little direct affect on the functionality models. Despite this, interactions were noted in the WMM (Table 13) and OEM systems (Table 14) and the WMM texture (Figure 24) in experiment 3. In experiment 4, there were no significant differences between diets on any of the functionality models (Section 6.3). Although previous work on this subject is restricted, the results here are in general agreement with Arafa *et al* (1985) and Ristic *et al* (1990) with regards to the texture of the meat. Ricard *et al* (1986) suggested an interaction between the diet and genotype with broiler-type birds producing more tender meat and layer-type birds producing tougher meat when fed a low energy diets. The results here are also in broad agreement with Grey *et al* (1986) who found similar results in turkeys. Here the results suggest that although broiler and layer-type birds are fed different diets, the layer-type diet would not adversely affect the meat processing capability if the meat was comminuted with salts and phosphates, reformed and cooked.

The processing factors had a far more significant effect on the functional properties of the meat than the animal production factors. The post-incubation pH, soaked, cooked yield and texture were significantly affected by the brine system used in all experiments.

One of the mechanisms for the processing factors improving the functional properties of meat is by increasing the extraction of myofibrillar proteins. The role of processing factors on cytoskeletal proteins particularly titin, the most abundant protein, is little understood. Titin's role in the functional properties of meat still remains some what of an enigma. Only the microscopic evidence here suggests some function in the WMM (Figure 32, A, B, C and D). However, by itself, extraction of myofibrillar proteins, appears to have a minor role, as extracted myofibrillar proteins have very poor functional properties if they are heated in the absence of salts and/ or phosphates (Macfarlane *et al*, 1977; Siegel and Schmidt, 1979b) and experiments 1, 2, 3 and 4 controls. Traditionally, changes in functional properties of meat proteins when salts

and phosphates are added have been attributed to changes in the pH and /or ionic strength, which explains some but not all of the changes in functionality, this is particularly evident from Figure 8 and Figure 9 although the same pattern can be seen with the WMM soaked and cooked yield for experiments 1, 2, 3 and 4. For example, the extent to which NaCl increases functionality was thought to be directly related to the increase in ionic strength produced by the salt (Hamm, 1970). Likewise, the extent to which phosphates were thought to improve functionality was directly related to their effect on ionic strength and pH (Trout and Schmidt, 1984). Ionic strength does not completely explain the salt-induced changes in meat protein functionality, because ionic strength relates to the change in electrostatic interactions that stabilise the muscle protein structure. Several authors have pointed out that salts only affect these electrostatic interactions when the ionic strength is below 0.1 (von Hippel and Schleich, 1969; Franks and Eagland, 1975; Melander and Horvath, 1977). At ionic strengths greater than 0.1, the layer of ions surrounding the charged groups on proteins is increased to the point where it shields the charged groups and prevents them from interacting electrostatically with each other. These authors also point out that the changes in protein conformation that occur at ionic strengths greater than 0.1 are due to the changes in hydrophobic interactions, which are major forces stabilising protein structure because they provide a centre for folding, through the indirect effect of their ceasing to disrupt the water structure (Friedli, 2003). They also affect the structure in a highly specific manner because their varied sizes and shapes fit together in very efficient packing order (Friedli, 2003). So electrostatic interactions are most influenced by salts when the ionic strength is below 0.1; above 0.1 hydrophobic forces become more important

What has not been explained, however, is why phosphates improve functionality more than other salts of comparable ionic strength and pH, (Figure 8 and Figure 9) or why they only produce this greater effect when the sodium chloride concentration in the product is higher than 0.8 % (Bendall, 1954; Hellendorn, 1962). First, the extent to which the phosphates increase functionality depends on the type and, possibly, the molar concentration of phosphate. If these changes in functionality were due entirely to changes in electrostatic interactions, then all treatments at the same ionic strength (including sodium chloride) should have increased functionality to the same extent; however this was not the case here (Figure 8 and Figure 9).

Second, in experiments 1, 2, 3 and 4, phosphates had essentially the same effect on functionality as sodium chloride, but as the ionic strength increased, their effectiveness relative to the sodium chloride treatment increased. Thus, at lower ionic strength, phosphates may affect protein functionality mainly by changing electrostatic bonds (Melander and Horvath, 1977). However, these electrostatically induced changes in conformation actually decrease the functional properties of meat proteins (Trout and Schmidt, 1984). Increasing the ionic strength reduces the electrostatic effects of the phosphates. This reduction occurs partly because of the decreased ions shielding (Section 1.3.2) and partly because of the charge on the phosphates, in that the higher sodium ion concentration associated with the higher ionic strength reduces the degree of dissociation of sodium phosphates and hence reduces the charge on the phosphate ions (Glasstone and Lewis, 1970). Once the electrostatic effects of the phosphates are reduced, the hydrophobic effects of the phosphates become dominant. Presumably, it is the hydrophobic effects of phosphates that increase the functional properties of meat proteins.

Third, within all the experiments there was an increase in the pH of the meat post-incubation. This increase in meat pH on the one hand would probably increase the charge on both the meat proteins and the phosphates, and changing electrostatic interactions promoting the general dispersion of myofibrillar proteins (Offer *et al.*, 1989a). The increase in the meat pH would also facilitate the dispersal of sarcoplasmic proteins. Monin and Laborde (2003) showed a pH-dependent interaction between myofibrils and sarcoplasmic compounds, which resulted in higher water holding capacity at pH values removed from the isoelectric point. Certainly there is evidence that a sarcoplasmic precipitate is found on myofibrillar proteins (Bendall and Wismer-Pedersen, 1962; Scopes and Lawrie, 1963; Voyle, 1979). The microscopic evidence shown here (Figure 32 A and B) showed some obscuring of the myofilaments, which may have been caused by sarcoplasmic proteins associating with the myofibrils. The electrophoresis results presented here (Section 6.7) support the theory that with an increase in the meat pH, there is a general dispersion of actin and myosin proteins. This general dispersion was further emphasised when salt and phosphate was added together (Figure 35 B3; Figure 36 B3; Figure 37 B6). So, when salt is added to meat



the sarcoplasmic proteins form a complex with the myofibrillar proteins, the sarcoplasmic proteins then inhibit the myofibrillar proteins from being extracted. The addition of phosphates loosens the sarcoplasmic proteins allowing the salt to interact with the myofibrillar proteins. This evidence concurs with many others suggesting that when salts and phosphates are added together they have a synergistic affect.

In summary the increases in functionality produced by salts was additive. When phosphates are used with salts the effects are not directly additive but are synergistic. The presence of salts increases the effectiveness of phosphates. With addition of salt and phosphate together there is an increase in the meat pH. This occurs because the increase in functionality produced by pH is additive with that produced by the salt and phosphate.

### Microscopy

The use of CLSM in the field of meat science is still relatively novel and provides the capability to optically section through samples and develop 3D images of the internal structures of the meat emulsions (Section 6.6) with minimal sample disturbance. This is of great benefit when examining shear-sensitive samples such as meat and meat products, where other microscopy techniques would involve physical disruption of the sample. Although the CLSM provided useful information on the internal structures (CMM and OEM), the CLSM afforded limited visual information with regards to the WMM. This said, the main proteins contained within the A/I overlap are actomyosin and titin (Section 1.1.1) and it would seem likely that these proteins constitute the dark bands (Figure 32 A, B, C and D). Titin can interact with myosin and actin, and may enhance precipitation of actomyosin. These micrographs suggest that the dark bands derived from the A/I overlap, and consist mainly of denatured actomyosin and titin. The remaining sarcomere proteins are probably dispersed through the meat structure in the form of a water-holding gel. However, as the micrographs are not that clear, what is evident is that NaCl, and combined NaCl and  $\text{Na}_5\text{P}_3\text{O}_{10}$ , promote the general dispersion of myofibrillar proteins; and in this case the A/I overlap regions rich in actomyosin would seem most resistant to dispersion.

Research based on microscopic observations of comminuted meats led to two different theories about their stability: fat emulsification (Hansen, 1960) and physical entrapment of fat in a protein matrix (Van den Oord and Visser, 1973). However, the results here suggest that the microstructure of a comminuted meat system cannot be explained by one theory alone. Moreover, the importance of fat emulsification versus physical restriction of fat coalescence may vary with different processing conditions (Smith, 1988). The results here concur with the current view that a comminuted meat product represents a complex multiphase system consisting of solid meat pieces, a gel and an emulsion (Writh, 1985; Hermansson, 1988).

From observations of the emulsions (CMM) after texture measurement, three failure mechanisms were identified (Figure 21). Failure mechanisms 2 (meat pieces splitting) and 3 (cleavage of two opposing pieces of connective tissue) can be explained by the work reported by Donnelly and Purslow (1987). Failure mechanism 1 (gel failure) could be partly explained by the microscopic observations. It seems likely that void size and porosity, as well as degrees of inter-linking (Section 6.6), are the cause of gel failure, in that gels with larger voids, numerous pores, and modest interlinking (Figure 34 A) fail at lower mean peak loads.

Due to equipment failure, some interesting areas of study were never pursued in this project. Further CLSM work could have determined what role, if any, titin has in processed meat products. Although much is known of its weight and structure, its role in meat and meat products is still speculative. Using the 3 D capability of the CLSM, models could have been developed to track added metal ions, such as sodium, through meat emulsions determining if they have predilection sites. The 3 D capability could also be a powerful tool to understand how structural information relates to mechanical and sensory information.

This project has demonstrated that the use of layer-type birds for meat and meat products is a possibility. It is unlikely that layer-type meat products would provide products at the higher end of the market such as fresh kiev's or restructured drumsticks. Layer-type meat would most likely provide products at the lower value market and possibly the food service sector such as grillsteaks or breaded products. Furthermore, as is common with comminuted meat products, a mixture of broiler-type

and layer-type meat could provide an acceptable product. Doubtless the ratio of broiler to layer-type meat, muscle type, pH of brine system, extent of protein to protein interaction in the meat, and degree of comminution would be important factors in the formation of these products. It must be emphasised that other differences, such as connective tissue organisation, biochemical characteristics of muscle fibres and differences in chemical structure of myosin and actin, may also have some bearing on the meat processing behaviour. Control of these features is, however, rather more in the area of genetics, whilst pH adjustment is an operation which can be undertaken in a meat factory.

## Appendix A

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Chicks from each genotype (broiler-type and layer-type) were wing-banded at day old, weighed, divided into groups.. Each group was placed in one of 32 floor pens (sawdust litter) divided across four rooms with two blocks in each room. The broiler-type and layer-type birds were fed either a high quality (HQ) or a low quality (LQ) diet giving four treatment groups (Broiler-type HQ, Broiler-type LQ, Layer-type HQ and Layer-type LQ). Each treatment contained 8 replicates (2 x 2 factorial design).

### *General husbandry & feed formulation*

Each of the rooms was lit with 4 60-watt normal bulbs and standard commercial lighting practice was employed (23h light: 1h dark) across all treatment groups. Initial room temperature on day one was 30°C and thereafter was gradually reduced until 21 d from which point the temperature was maintained at 21°C. Temperature varied slightly over the course of the experiment ( $\pm 2^\circ\text{C}$ ) due to the nature of the heating system and time of year. Pen dimensions were in line with stocking density requirements as defined by DEFRA (2000).

Birds were supplied with water and feed *ad libitum*, feed was formulated and mixed in-house and provided in mash form. Table 25 shows the standard management diet. Rations were formulated using the UNEform feed formulation package (Ross Breeders, 1996). The HQ diet was formulated to reflect the commercial standard in terms of metabolisable energy (ME) and crude protein (CP). The LQ diet was formulated to be ~10% lower in nutritional value by dilution with oat feed, to give a similar nutrient level to the 1972 broiler diet, based on available data from the 1972 broiler production manual (Ross Breeders, 1996).

**Table 25** *Standard Management Diet*

Dietary Ingredients g/kg	Starter	Grower	Finisher
Corn	517.78	492.25	550.74
Wheat	-	60.0	50
Corn protein supplement	-	84.5	55.16
Soybean	180.0	246.1	183.50
Soybean meal	241.6	74.83	99.83
Fish meal	19.83	3.33	-
Tallow	5.43	-	20
Limestone	8.45	9.45	9.55
Salt	2.73	2.91	5.0
DCP	18.33	19.48	19.13
Vitamin premix <sup>1</sup>	2.00	2.00	2.0
Mineral premix <sup>2</sup>	1.00	1.00	1.0
L-lysine	-	2.3	2.03
DL-methionine	2.85	1.85	2.06
Total	1000	1000	1000
Nutrient Analysis			
Dry matter, g/kg	886.0	884.3	890.8
Crude Protein, g/kg	244.7	236.0	207.4
Metabolisable Energy, kcal/kg	3054	3159	3200
Crude cellulose, g/kg	29.9	39.5	33.87
Crude fat, g/kg	60.87	59.2	61.3
Starch, g/kg	391.3	429.3	379.5

<sup>1</sup>Containing 4.800 IU Vit A (retinyl palmitate); 600.000 IU Vit D (cholecalciferol); 12.000 mg Vit E (dl-"-tocopherol acetate); 2.000 mg Vit K , 1.200 mg Vit B , 2.400 mg Vit B , 2.000 mg Vit B , 12 mg Vit B , 16.000 mg Nicotinamide, 4.000 mg Calcium-D-Pantothenate, 300 mg Folic acid, 30 mg D-Biotin, 150.000 mg Choline chloride, 4.000 mg Antioxidant. <sup>2</sup> Containing 80.000 mg Mn; 80.000 mg Fe; 60.000 mg Zn; 8.000 mg Cu; 500 mg I; 200 mg Co; and 150 mg Se.

Table 26 describes the diet formulation and the inclusion level of each of the dietary ingredients in g kg<sup>-1</sup>. Table 27 and 31 supply a detailed list of dietary requirements and estimated levels of their inclusion in each ration. The two columns (required and supplied) represent the ideal inclusion levels of each dietary component and the calculated level of each dietary component as estimated by the UNEform feed formulation package. Estimates of dry matter content (DM), gross energy of the diet (MJ kg<sup>-1</sup>), crude protein (g kg<sup>-1</sup>) and the mineral content of the grower and finisher rations are supplied in Table 28. The nutritional specifications of the HQ rations were as follows: Starter: 0-10 days (22-24% crude protein (CP); 12.6 MJ kg<sup>-1</sup> ME);

Grower: 11-24 days (20-22% CP; 13.3 MJ kg<sup>-1</sup> ME) and Finisher: 25 days slaughter (17-19% CP; 13.5 MJ kg<sup>-1</sup> ME). The ME and CP levels of the LQ rations were as follows; Starter (19.87% CP; 11.18 MJ kg<sup>-1</sup> ME); Grower (19.13% CP; 11.68 MJ kg<sup>-1</sup> ME); Finisher (17.12% CP; 11.86 MJ kg<sup>-1</sup> ME).

**Table 26** Diet formulation used for experiments 3 and 4.

Ingredient	Ration					
	HQ Starter	LQ Starter	HQ Grower	LQ Grower	HQ Finish	LQ Finish
Units	g kg <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>	g kg <sup>-1</sup>
Wheat	497.5	166	520	486	550	515
Oats	46	201	0	0	0	0
Safflower oil	12	1	50	10	40	25
FF Soya	145	190	104	120	100	150
Soybean meal	235	148	231	130	160	40
Maize	0	130	0	60	50	55
Monodical	15	15	15	15	15	15
Limestone	15	15	15	15	15	15
NaCl	3.5	3.5	3.5	3.5	3.5	3.5
Lysine	3	2.5	3	2.5	3.0	2.5
Methionine	3	3	3.5	3	3.5	3
Oat feed	20	120	40	130	3.5	141
Fishmeal	0	0	10	20	20	30
*Premix	5	5	5	5	5	5
TOTAL	1000	1000	1000	1000	1000	1000

\*Vitamin and mineral premix provided (units g kg<sup>-1</sup>): Vit A 12,000 iu; Vit D3 5,000 iu; Vit E 50 iu; Vit K 3 mg; Vit B2 7 mg; Vit B6 5 mg; Vit B12 15 µg; Nicotinic acid 50 mg; Pantothenic acid 15 mg; Folic acid 1 mg; Biotin 200 µg; Iron 80 mg; Copper 10mg; Manganese 100 mg; Cobalt 0.5 mg; Zinc 8 mg; Iodine 1 mg; Selenium 0.2 mg; Molybdenum 0.5 mg.

**Table 27** *Low Quality Rations*

Nutrient	Units**	Starter		Grower		Finisher	
		Required	Supplied	Required	Supplied	Required	Supplied
ME	MJ kg <sup>-1</sup>	11	111.8	11.5	11.68	12	11.86
Crude Protein (CP)	g kg <sup>-1</sup>	200	198.7	190	191.3	170	171.2
Crude Fat	g kg <sup>-1</sup>	50	77	56	52.2	56	67
Fibre	g kg <sup>-1</sup>	60	74	40	60.1	40	66
Linoleic acid	g kg <sup>-1</sup>	11	32.6	10	25.5	10	32.6
Lysine	g kg <sup>-1</sup>	9.9	13.3	12	12.6	11	10.8
Methionine	g kg <sup>-1</sup>	4.7	5.2	4.8	5.3	4	4.7
Met+Cys	g kg <sup>-1</sup>	6.9	8.2	9.4	8.4	8.5	7.5
Arginine	g kg <sup>-1</sup>	11	13.6	9	12.2	8.5	10.7
Cystine	g kg <sup>-1</sup>	4	3.3	3.9	3.1	3.7	2.8
IsoLeucine	g kg <sup>-1</sup>	8	9.2	7.5	9.3	7	7.8
Tryptophan	g kg <sup>-1</sup>	2	2.6	2.1	2.2	1.9	2.0
Tyrosine	g kg <sup>-1</sup>	6	7.4	5	7.4	5	6.3
Valine	g kg <sup>-1</sup>	9	8.5	9	8.1	8.5	7.1
Calcium	g kg <sup>-1</sup>	9.5	9.9	9	10.8	8.5	9.7
Total Phosphorus	g kg <sup>-1</sup>	7	6.2	6	7.1	6	6.3
Avail. Phosphorus	g kg <sup>-1</sup>	4.3	4.5	4.8	5.1	4.4	4.5
Sodium	g kg <sup>-1</sup>	1.7	1.8	1.7	2.0	1.7	1.9
Manganese	mg kg <sup>-1</sup>	100	58	100	216	100	57
Zinc	mg kg <sup>-1</sup>	80	39	80	135	60	37
Vitamin E	mg kg <sup>-1</sup>	50	100	50	500	50	100
Calcium:Avail.P		1.9	2.22	1.8	2.13	1.93	2.19
ME:CP		548	562.82	605	610.77	675	692.71

ME: Metabolisable energy; P: Phosphorus

**Table 28** High Quality Rations

Nutrient	Units	Starter		Grower		Finisher	
		Required	Supplied	Required	Supplied	Required	Supplied
ME	MJ kg <sup>-1</sup>	12.60	12.61	13.30	13.29	13.50	13.37
Crude Protein (CP)	g kg <sup>-1</sup>	230	231.9	220	220.5	200	200.2
Crude Fat	g kg <sup>-1</sup>	50	62.0	70	89.0	70.0	81.0
Fibre	g kg <sup>-1</sup>	30	37.7	30	37.2	30.0	37.7
Linoleic acid	g kg <sup>-1</sup>	12.5	28.8	12.0	52.5	10.0	43.9
Lysine	g kg <sup>-1</sup>	13.6	16.1	13.0	15.4	11.3	13.4
Methionine	g kg <sup>-1</sup>	5.3	5.8	5.2	6.1	4.7	5.7
Met+Cys	g kg <sup>-1</sup>	9.8	9.7	9.4	9.8	8.5	9.0
Arginine	g kg <sup>-1</sup>	12.5	16.2	9.5	15.2	9.0	13.0
Cystine	g kg <sup>-1</sup>	4.5	3.9	4.2	3.7	3.8	3.3
IsoLeucine	g kg <sup>-1</sup>	9	11.8	8.0	11.3	7.5	9.7
Tryptophan	g kg <sup>-1</sup>	2.3	2.9	2.1	2.6	1.9	2.4
Tyrosine	g kg <sup>-1</sup>	6	9.3	5.0	8.9	5	7.7
Valine	g kg <sup>-1</sup>	9	10	9.0	9.4	8.5	8.4
Calcium	g kg <sup>-1</sup>	9.5	10	9	10.4	8.5	10.2
Total Phosphorus	g kg <sup>-1</sup>	7	7.4	6	7.6	6	7.4
Avail. Phosphorus	g kg <sup>-1</sup>	5	4.7	4.8	4.9	4.4	4.8
Sodium	g kg <sup>-1</sup>	1.7	1.9	1.7	1.9	1.7	2.0
Manganese	mg kg <sup>-1</sup>	100	61	100	220	100	62
Zinc	mg kg <sup>-1</sup>	80	43	80	139	60	42
Vitamin E	mg kg <sup>-1</sup>	50	100	50	500	50	100
Calcium:Avail.P		1.90	2.13	1.80	2.12	1.93	2.11
ME:CP		548	543.16	605	602.65	675	667.88

ME: Metabolisable energy; P: Phosphorus



**Table 29** Actual LQ and HQ Grower and Finisher – Actual chemical composition

Determination - Units	HQGrower	LQGrower	HQFinisher	LQFinisher
Dry Matter g kg <sup>-1</sup>	876	878	873	877
CP g kg <sup>-1</sup> DM	214	202	226	194
Gross Energy MJ kg <sup>-1</sup> DM	17.86	17.09	18.05	17.60
Phosphorus g kg <sup>-1</sup> DM	9.87	9.18	9.32	8.76
Potassium g kg <sup>-1</sup> DM	10.6	8.5	10	8.1
Sodium g kg <sup>-1</sup> DM	2.1	2.1	2	1.8
Magnesium g kg <sup>-1</sup> DM	1.9	1.6	1.8	1.5
Calcium g kg <sup>-1</sup> DM	15.8	15.4	15.4	14.1
Sulphur g kg <sup>-1</sup> DM	3.8	3.2	3.5	3.1
Boron mg kg <sup>-1</sup> DM	14.9	9.57	13.4	9.52
Copper mg kg <sup>-1</sup> DM	46.3	20.6	24.2	22.4
Iron mg kg <sup>-1</sup> DM	255	327	247	247
Manganese mg kg <sup>-1</sup> DM	114	138	117	105
Zinc mg kg <sup>-1</sup> DM	117	124	113	103
Total minerals g kg <sup>-1</sup> DM	132.22	128.40	129.83	125.55

Given the different growth architecture of the two genotypes on different dietary regimens, the feeding period for each ration was staggered between the two groups of birds. Using the Layer-type HQ as the benchmark on which to base time of ration changeover, the feeding program was as follows: diet changeover at same ages for broiler-type HQ and broiler-type LQ, diet changeover at same BM for broiler-type HQ and layer-type HQ and diet changeover at same age for layer-type HQ and layer-type LQ. The layer-type used in this study is given 10 weeks to reach market weight (i.e. the same body mass as the broiler-type at 42 days) and therefore, required a longer period on each of the rations than the layer-type to reach optimum weight at diet changeover Table 30.

**Table 30** *Feeding Program*

<b>Broiler-type HQ</b>			
Days	0-10	11-24	25-kill
Ration	Starter	Grower	Finisher
Predicted BM (gms)	250	900	2110
Estimated feed (kgs)	60	180	456
<b>Broilers-type LQ</b>			
Days	0-10	11-24	25-kill
Ration	Starter	Grower	Finisher
Estimated BM (gms)	200	750	1900
Estimated feed (kgs)	84	210	501.6
<b>Layer-type HQ</b>			
Days	0-16	16-35	36-kill
Ration	Starter	Grower	Finisher
Estimated BM (gms)	250	980	2110
Estimated feed (kgs)	84	180	456
<b>Layer-type LQ</b>			
Days	0-16	16-35	36-kill
Ration	Starter	Grower	Finisher
Estimated BM (gms)	220	850	1900
Estimated feed (kgs)	84	198	501.6

Feed was withdrawn for 12 hours prior to sacrifice, in line with standard commercial procedures. At the end of the experimental period birds were stunned and exsanguinated at the Carcass Evaluation Unit (CEU) SAC, Ayr.

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**Appendix B**


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**Table 31 Concentrations (ml) of NaCl, NaOH, HCl and H<sub>2</sub>O concentrations for the pilot pH study.**

System (ml)	1	2	3	4	5	6	7	8	9	10	11	12
NaCl	9.0	9.5	10	10.5	11	11.5	11.7	12.2	12.5	12.2	11.7	11.5
NaOH	3.5	3.0	2.5	2.0	1.5	1.0	0.75	0.25	0	0	0	0.0
HCl	0	0	0	0	0	0	0	0	0	0.25	0.75	1.0
H <sub>2</sub> O	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5	37.5

System (ml)	13	14	15	16	17	18
NaCl	11.0	10.5	10.0	9.5	9.0	8.5
NaOH	0	0	0	0	0	0
HCl	1.5	2.0	2.5	3.0	3.5	4.0
H <sub>2</sub> O	37.5	37.5	37.5	37.5	37.5	37.5

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