COLLAGEN SOLUBILITY OF A-MATURITY BOVINE *LONGISSIMUS* MUSCLE AS AFFECTED BY NUTRITIONAL REGIMEN

by

JAMES BRIAN HALL

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Approved by:

Melvin C Hunt
Major Professor

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Chapter I

General Introduction

Palatability is a major factor that influences consumer acceptance of beef. Meat palatability is a highly researched topic, and has been found to depend mainly on tenderness, juiciness and flavor, with tenderness being the most important factor. Muscle tenderness appears to be a two-component system composed of muscle fiber tenderness, and connective tissue tenderness.

Generally, quantitative differences in the connective tissue component, comprised mainly of collagen, are thought to account for tenderness differences between muscles while qualitative differences in collagen accounts for some of the variation in tenderness between animal age groups. The myofibrillar component generally is more important in young cattle where connective tissue crosslinking has not yet resulted in tough connective tissue. Cold shortening of the myofibrillar component can affect tenderness. As yet unexplained, however, are the variations in tenderness of muscles from young animals with similar sarcomere length. Intramuscular connective tissue may influence tenderness, even in young animals, particularly those fed on widely varing nutritional regimens.

Although the specific effect of diet energy density on the maturation of connective tissue is not well known, there is evidence that diet energy density has some effect on collagen solubility. Grass finished cattle are frequently less tender than grain finished cattle. Some studies have related this difference to less marbling and

subcutaneous fat thereby decreasing the insulatory effect of these components on the muscle fibers which leads to increased cold shortening during postmortem chilling (Smith et al., 1974). More recently, Smith et al. (1977, 1979) have shown that grass-fed cattle finished on a high concentrate diet for as little as 49 to 98 days, are at least as tender as cattle finished on more conventional concentrate feeding diets. Therefore molecular changes in collagen may be an important factor involved in tenderness of young cattle.

The purpose of this study was to determine the effect of widely different diet energy densities on skeletal muscle collagen solubility of young animals, and to relate collagen solubility to the tenderness of the longissimus muscle.

Chapter II

Muscle Connective Tissue - General Description

Connective tissue, found throughout the body, connects other tissues, provides a framework, and supports the entire body by means of cartilage and bones (Dellmann et al., 1976). It further serves many vital purposes in life, by giving support to muscle, providing a framework for blood vessels and nerves, and protecting the contractile muscle structure from damage by over-extension.

The importance connective tissue has on muscle tissue alone, is very great. Without it, muscle cells could not function or exist (Price et al., 1971). Every single muscle fiber is wrapped in a thin network of connective tissue called the endomysium. This same network functions to surround the muscle bundles with larger sheets known as the perimysium. Finally the network exceeds in encapsulating the entire muscle by enclosing it within the epimysium (Cassens, 1971; Meyer, 1968). These connective tissue sheaths come together at the end of the muscle to form tendons (Cassens, 1971).

Generally, connective tissues are characterized as having relatively few cells, and substantial amounts of extracellular substances varing in consistency from a soft jelly to a tough fibrous mass (Meyer, 1968). This extracellular substance contains fibers embedded in it thus providing the structural elements of the connective tissue. The extracellular substance in cartilage has an elastic consistency; in bone it is tough and impregnated with calcium salts; and in blood and lymph there are no fibers and the extracellular substance is fluid (Forrest et al., 1975).

Connective Tissue Proper

Connective tissue proper is made up of a structureless mass known as the ground substance, in which the cells and extracellular fibers are embedded.

Since the connective tissue proper is characterized by presence of distinct extracellular fibers it is commonly refered to as fibrous connective tissue. Supportive connective tissue is made up of bone and cartilage which other tissues attach to, thus providing the body with structural support. These two classes of connective tissue possess many similarities in composition and function and, in fact, fibers of the connective tissue proper are sometimes continuous with bone and cartilage (Forrest et al., 1975).

The connective tissue proper contains two different types of cells known either as fixed or wandering cells. Fixed cells include fibroblasts, undifferentiated mesenchyme cells, and specialized adipose cells. Wandering cells are those that move around the body and include lymph cells, free macrophages, plasma cells, mast cells, and eosinophils (Price et al., 1971; Forrest et al., 1975).

Ground Substance

The ground substance is a homogeneous appearing background material of mucopolysaccharides and mucopolysaccharide-protein complexes in differing degrees of polymerization (Miller et al., 1956). It is a frequently viscous solution containing tropocollagen and tropoelastin, the precursors of collagen and elastin, respectively.

Hyaluronic acid and the chondroitin sulfates are important mucopolysaccharides contained in the ground substance (Price et al., 1971). Hyaluronic acid is very viscous and is found between connective tissue fibers and in synovial fluid. Chondroitin sulfates are found in

cartilage, tendons, and adult bone. Besides providing a barrier against infective agents, these two mucopolysaccharides and their associated proteins act as lubricants, intercellular cementing substances, and structural matter in bone and cartilage (Forrest et al., 1975).

Extracellular Fibers

Extracellular connective tissue fibers include three types: collagen, elastin, and reticulin (Dellmann et al., 1976). These fibers commonly are arranged in two basic structural orientations referred to as either loose connective tissue or dense connective tissue.

Loose connective tissue, the most common type in the adult animal, is irregularly arranged because of the small spaces between the cells and fibers. Loose connective tissue is found around blood vessels and nerves, between muscle bundles and the layers of smooth musculature of hollow organs. Its functions are varied and range from support in various locations to participation in tissue repair, bodily defense, and water metabolism (Dellmann et al., 1976).

Dense connective tissue commonly is classified by fiber arrangement. Dense regular connective tissue is more specialized in function than other connective tissues, and has, therefore, lost its potential for varying functional differentiation (Dellmann et al., 1976). The fibers of dense regular connective tissue are arranged in bundles lying parallel to each other and occur as collagen tendons and ligaments and elastin ligaments (Forrest et al., 1975). Dense irregular connective tissue consists of fibers densely interwoven but in a random arrangement. Therefore, they possess characteristics of both loose and dense regular connective tissue. The collagen fibers that predominate this classification are generally arranged in bundles that cross each other at different

angles, and at times through different planes, thus allowing, for example, changes in the size of an organ or the diameter of a muscle (Dellmann et al., 1976). This also adds to the strength of these structures and protects them from over-extension.

Collagenous Connective Tissue

Collagen is the principal structure protein in connective tissue and is the bodys most abundant protein contributing 25 to 40% (McClain, 1977) of the total body proteins. Collagen is classified as a glycoprotein (Spiro, 1970) because it is highly complexed with the carbohydrates galactose and glucose. One-third of the total amino acid content of collagen is glycine, and hydroxyproline and proline make up nearly another one-third (Table 1). Hydroxyproline is unique to this animal protein in that the concentration in collagen is relatively constant at 13 to 14% of the total amino acids. Because of this uniqueness, chemical determinations of hydroxyproline are used to quantitate collagen in tissues.

Collagen is the primary connective tissue in skin, bone, tendon, cartilage,and is a vital component of the cardiovascular system (Price et al., 1971). It comprises the protein matrix for the deposition of depot fats, and supports and surrounds individual myofibers (endomysium), muscle bundles (perimysium) and the entire muscle (epimysium). Although abundant throughout the body, collagen concentrations vary among skeletal muscles with concentrations being greater in muscles enduring greater physical activity (Forrest et al., 1975).

The properties of collagen that allow it to act as the supportive framework are dependent upon the high structural stability of the molecule (McClain, 1976). This stability is maintained through its

unique molecular configuration, highly specific alignment of the molecules during extracellular aggregation, and most importantly, by the formation of covalent crosslinkages that give the fibers their high tensile strength and resistance to chemical attack (McClain, 1976). Additionally, McClain (1977) states that collagen is the only protein in the body that undergoes molecular changes with advancing age.

Elastic Connective Tissue

Most elastic fibers are relatively thick and generally occur as individual, refractile, branching fibers. They are characterized by their chemical insolubility. Upon exhaustive extraction with dilute acids or alkali they yield elastin (Hall, 1961). Elastin is located in the amorphous substance of elastic fibers and is *per se* a rubbery protein present mainly in ligaments and arterial walls (Dellmann *et al.*, 1976). Elastin is a relatively minor component in mammalian skin, tendon, adipose tissue and muscle (Bodwell and McClain, 1971).

The elastin content in skeletal muscles has not been closely associated with meat tenderness (Cross *et al.*, 1973). The elastin structure is different than collagen. Bovine elastin contains 14% more nonpolar amino acids than bovine collagen; however, the quantities of histidine, cystine, tyrosine, and tryptophan are similar for collagen and elastin (Table 1) (Meyer, 1968). Paul (1972), reported collagen contains 50 to 100 times as much hydroxyproline as elastin.

Desmosine and isodesmosine are two unique amino acids making up eight amino acid residues per 1000 in elastin. The extreme insolubility of elastin is due largely to its high content (90%) of these types of nonpolar amino acids. The desmosine crosslinkages that bind four adjacent

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TABLE I. PRINCIPAL AMINO ACIDS IN RETICULIN, ELASTIN AND COLLAGEN

Amino Acid	Human Kidney Reticular[a]	Bovine Ligamentum Nuchea Elastin[b]	Bovine Intramuscular Collagen[c]
Glycine	31*	33	33
Proline	10	11	12
Hydroxyproline	11	1	11-14
Hydroxylysine	1	-	1
Alanine	10	22	10
Tryptophan	-	Very Low	-
Desmosine Isodesmosine	-	1	-
Tyrosine, Histidine, Cystine & Tryptophan	1	1	1
Polar Residues	-	5	18

* % of total amino acid residues

[a] Lowther (1963)

[b] Gotte et al. (1963)

[c] McClain et al. (1971)

elastin chains together has a large effect also on this insolubility (Forrest et al., 1975).

Reticular Connective Tissue

Reticulin occurs at the lowest percentage of connective tissue components of muscle tissue and is also the least understood. Reticulin fibers are small and form delicate, flexible supportive networks around capillaries, muscle fibers, nerves, adipose cells, and lymphatic organs. Reticulin is similar structually to individual collagen fibrils (Jackson et al., 1956). The variations in structure include the many branched processes and large nucleus of the reticulin fibers. The ability to differentiate between collagen and reticulin fibers depends on the ability of reticulin to react with silver stains (Hall, 1961). Dellmann et al., (1976) indicates that reticulin is considered the forerunner of collagen fibers because when individual reticular fibers are bound together, they form a collagen fiber which no longer reacts with heavy metal ions. Swatland (1976) says the differences in physical and staining properties between reticular fibers and small diameter collagen fibers are due probably to three factors: (1) the arrangement of fibrils within the fiber, (2) the type of matrix between the fibrils, and (3) the nature of the lipids associated with the reticular fibers (Pearse, 1968). The exact nature of reticular fibers per se and their importance to meat textural properties is unknown.

Adipose Connective Tissue

Adipose tissue is considered a special form of reticular connective tissue (Dellmann et al., 1976). Recent research has shown adipose tissue

develops from dense groups of highly vascularized reticular connective tissue cells in which fat deposits occur (Forrest et al., 1975).

Most mammals contain two types of adipose tissue, white and brown. White adipose tissue predominates in the bodies of meat animals. These cells are large and spherical in shape and are occupied almost entirely by one lipid droplet. Unlike other connective tissue the ground substance is scarce, and the cells are the main components of the tissue. Brown adipose tissue contains fat cells which are usually smaller in size, and is present in all these animals at birth but usually disappears or is converted to white adipose tissue within a few weeks of birth.

Besides being in adipose tissue, fat cells may also be scattered through, or in groups, in loose connective tissue (Meyer, 1968).

Since collagen is the most abundant form of connective tissue throughout the body and especially in muscle tissue, the remainder of this review will deal exclusively with collagen and its effects on meat texture.

Molecular Structure of Collagen

Traub et al. (1971), and Gallop et al. (1972) were among the first researchers to report that native collagen fibers are formed through a process of linear and lateral aggregation of thin, highly elongated macromolecules called tropocollagen. These macromolecules, are rod-like structures with a diameter of about 1.4 nm, a length of about 280 nm, and a molecular weight of about 300,000 daltons (Bodwell and McClain, 1971).

The tropocollagen molecule has three polypeptide chains, known as α chains, each of which contains approximately 1,000 amino acid residues and has a molecular weight of 100,000 daltons. Each α chain is coiled in a left-handed helix, and the three α chains in each tropocollagen molecule are coiled in a right-handed superhelix (Figure 1) (Ramachandran, 1967). This conformation provides some protection against chemical or enzymatic disruption.

Heat denaturation of collagen disrupts the superhelical structure and results in random coiled α (monomer), β (dimer), and γ (trimer) components depending on the number and position of covalent crosslinks between α chains.

Two forms of α chains, α_1 and α_2, occur in a 2:1 ratio within tropocollagen molecules (Price et al., 1971). Various combinations of these two types of chains, depending on the position of the covalent crosslinks, leads to the β (dimer) and γ (trimer) components. Three types of dimers are possible. β_{11} and β_{12} dimers are formed by intramolecular crosslinkage of two α_1 chains and one α_1 and one α_2 chain, respectively. β_{22} dimers are formed only through intermolecular crosslinkages

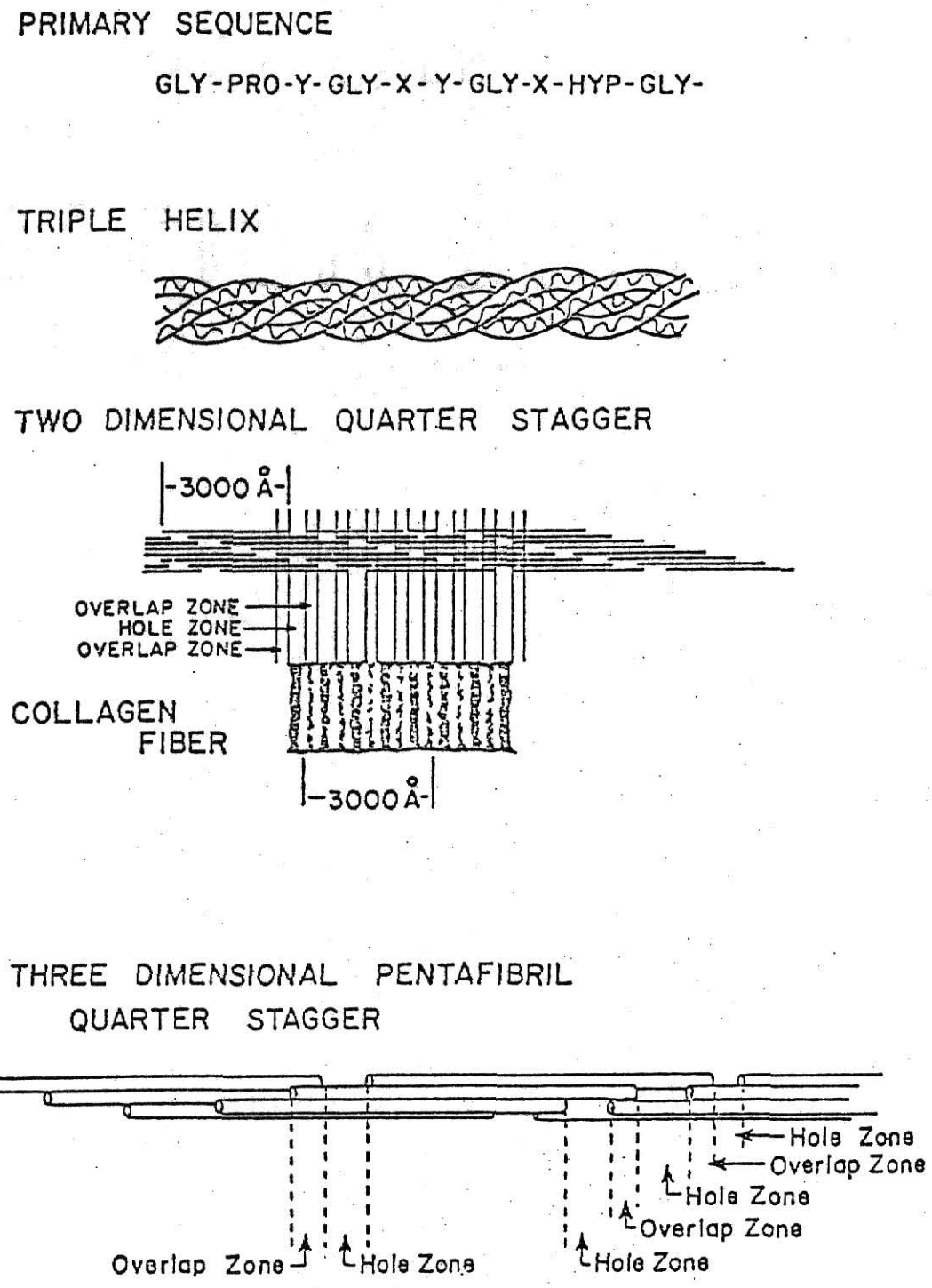

Figure 1. Schematic representation of tropocollagen structure (Dutson, 1976).

of two α_2 components. Four trimer components are possible. γ_{112} trimers result from intramolecular crosslinkages; γ_{111}, γ_{122}, and γ_{222} trimers are formed only through intermolecular crosslinkages (Price et al., 1971).

Price et al. (1971) indicate that α_1 and α_2 components behave differently. α_2 components have a greater potential for crosslinking than α_1 components, because α_2 monomers are: 1) more basic due to a higher histidine content, 2) higher in amino acids with hydrophobic side chains (valine, leucine, isoleucine) and, 3) lower in content of the imino acids proline and hydroxyproline.

A characteristic feature of the amino acid composition of collagen is the repeating tripeptide Gly-X-Y, with the X and Y frequently being proline and hydroxyproline, respectively. When proline is in the X position, the Y position is usually occupied by an amino acid other than hydroxyproline (Figure 1). Concurently when the Y position is occupied by hydroxyproline the X position is not proline (Traub et al., 1971). Proline and hydroxyproline are almost equal in proportion except in the regions at the N and C terminals, where hydroxyproline almost exclusively occurs in the Y position (Bailey and Robins, 1976).

Smith (1968) found that a collagen fibril consists of five tropocollagen molecules in the form of a pentafibril, assembled in a "quarter-stagger" formation (Figure 1). This formation is termed quarter-stagger because each tropocollagen molecule overlaps its neighboring molecule by three-fourths of its length. The hole and overlap regions caused by this formation account for the 650 Å periodicity of collagen fibrils. Due to this specific arrangement of collagen, inter- and intra-molecular crosslinks are necessary to stabilize its structure and give it the high dimensional stability needed to function in living tissue (Dutson, 1976; Marsh, 1977).

The diameter of collagen fibrils varies from .3 to .4 μm while

collagen fiber diameter ranges from 1 to 12 μm depending on the number of fibrils in the parallel array (Bornstein, 1974).

Collagen Types

Four genetically distinct types of collagen composed of five different α chains, α_1(I), α_1(II), α_1(III), α_1(IV) and α_2 which vary slightly in amino acid composition have been identified in various tissues. Table 2 summarizes the four collagen types (Miller et al., 1974, Bailey and Robins, 1976; Martin et al., 1975). Miller et al. (1974) report that each of the four types has three α chains. In general, types I, II and III are aggregated in fibrous form while type IV is an amorphous form in basement membranes (Bailey and Robins, 1976). Each of the four collagen types has characteristic α_1 chains, different from the others in amino acid composition and sequence, carbohydrate content, and types of crosslinkages (Chein, 1975; McClain, 1974; Bailey and Sims, 1976; Dutson, 1976). Type I collagen is unique since it has an α_2 chain along with two identical α_1(I) chains (Piez, 1967).

Chemistry of Collagen Crosslinking

McClain (1976) states that the molecular stability of collagen is due mainly to the formation of covalent crosslinks which give the collagen fibers their high tensile strength and resistance to chemical attack.

The sequence of events leading to formation of covalent crosslinks occurs extracellularly. Tropocollagen molecules contain nonhelical portions at both the N and C terminal ends, which are cleaved by the enzyme, procollagen peptidase, (Lichtenstein et al., 1973) to form the functional collagen molecule, which itself has short nonhelical segments at the N and C terminal ends.

Two types of crosslinkages are known to occur: 1) intramolecular

TABLE 2. COLLAGEN TYPES[a]

Type	Tissue Location	Molecular Composition	Distinctive Features
I	Skin, Tendon, Bone, Muscle	$\{\alpha_1(I)\}_2\alpha_2$	α_2 chains, low carbohydrate
II	Cartilage, Intervertebral Discs	$\{\alpha_1(II)\}_3$	α_1 chains only, 10% carbohydrate
III	Fetal Skin, Cardiovascular System, Synovial Membrane, Cardiac and Skeletal Muscle	$\{\alpha_1(III)\}_3$	α_1 chains only, low carbohydrate, disulfide bonds
IV	Basement Membrane	$\{\alpha_1(IV)\}_3$	α_1 chains only, 15% carbohydrate, high in hydroxylysine and hydroxyproline

[a]Dutson (1976).

crosslinkages within the tropocollagen molecule, and 2) intermolecular crosslinkages between molecules in the fibers.

Traub et al. (1971) has shown that the intramolecular crosslinkages located in the short, nonhelical portion at the N terminal end of the collagen molecule, occur by joining α chains to produce the β_{11} and β_{12} dimers and three α chains to produce the γ_{112} trimer. McClain (1976) states the initial stage in the formation of the intramolecular crosslinkages involves the oxidative deamination of specific lysine or hydroxylysine residues in the nonhelical N-terminal region by a copper requiring enzyme, lysyloxidase. The final product of this reaction, called allysine, is the δ-semialdehyde of α -amino adipic acid (Gallop et al., 1972). Two allysine residues on adjacent chains can interact by aldol condensation to form an α, β-unsaturated aldehyde (McClain, 1976).

This aldol bond is very stable; however, it serves only to crosslink the chains within a molecule and does not account for any increase in stability of the collagen matrix. Marsh (1977) stated that intramolecular crosslinkages do not contribute significantly to meat toughness.

McClain (1976) contends the formation of intermolecular covalent crosslinks is the only logical explanation for the increasing insolubility and tensile strength of the collagen fibers over time. With increasing time, covalent intermolecular crosslinkages form between tropocollagen molecules eventually forming mature collagen fibers which subsequently crosslink to form the connective tissue network. With aging, this network reaches high mechanical stability and resistance to heat and chemical denaturation.

Four basic types of reduced crosslinked components are involved in intermolecular bonding. These components are: hydroxylysinonorleucine

(HLNL), dihydroxylysinonorleucine (DHLNL), histidinohydroxymerodesmosine (HHM), and lysinonorleucine (LNL). The first three are of major significance in stabilizing the collagen matrix (McClain, 1976).

Aldehydes produced by the oxidative deamination of lysine or hydroxylysine can interact with other lysine or hydroxylysine residues present in adjacent molecules to form a reduced Schiff's base type of intermolecular crosslink (Tanzer, 1973). HLNL was the first major reduced component to be isolated and characterized (Bailey et al., 1968). It is formed from either the condensation of lysine with hydroxyallysine or of hydroxylysine with allysine resulting in formation of the aldimine bond between two collagen molecules. The unreduced crosslink of dehydro HLNL from hydroxylysine and allysine is a labile crosslink destroyed by heat, dilute acids, and certain aminothiols (Bailey et al., 1970), while those derived from hydroxyallysine and lysine are stable to heat and dilute acids (Wu, 1978). DHLNL is the second major reduced crosslink (Tanzer et al., 1968) and is predominant in tendon, bone, and cartilage. It is a reduced Schiff's base product of hydroxylysine. The unreduced crosslink of dehydro DHLNL is very stable to heat and dilute acids. It has been confirmed that the unreduced crosslinks of dehydro DHLNL and dehydro HLNL from hydroxyallysine and lysine, are stabilized in that migration of the double bond could take place to form the stable keto form by undergoing a spontaneous Amadori rearrangement (McClain, 1976; Bailey et al., 1974). This would explain why these two Schiff's bases increase maximally during rapid animal growth and then slowly decrease to virtual absence at maturity while the collagen molecule maintains a thermally stable and non-reducible nature (Bailey et al., 1971).

The third major crosslink precursor, HHM, has recently been shown to have a structure capable of crosslinking three or four peptide chains (Tanzer et al., 1973). This occurs by two allysine condensed aldol condensation products, producing a more complex intermolecular linkage by combining with the ε-amino group of hydroxylysine, generating hydroxymerodesmosine (Tanzer et al., 1973). Two allysine condensed aldol condensation products also combine with the side chain of a histidine residue producing aldol histidine or combine with both histidine and hydroxylysine to give HHM (Tanzer, 1973). Bailey et al., (1970) designated this as "fraction C" when he detected it in bovine achilles tendon and found it to be a labile collagen crosslink.

The fourth crosslink component lysinonorleucine (LNL), is a reduced Schiff's base product of lysine and allysine. This crosslink is present in such small quantities that it makes no significant contribution to collagen stability (McClain, 1976).

Role of Hydroxyproline in Collagen Determination

Since collagen is the major component of connective tissue and most hydroxyproline in animal tissue is in collagen, determination of this imino acid has been used to estimate collagen in bovine muscle tissue (Neuman and Logan, 1950; Stegeman, 1958; Prockop et al., 1960; Woesner, 1961; Dahl et al., 1963; Bergman and Loxley, 1963; Serafini - Cessi et al., 1964; Blumenkrantz et al., 1975).

Since Neuman and Logan (1950) developed a method for the spectrophotometric estimation of hydroxyproline, numerous other researchers have reported improved methods. Basically, hydroxyproline spectrophotometric methods are based on the oxidation of hydroxyproline to pyrrole-2-carboxylic acid, and the subsequent condensation of this intermediate compound with

Ehrlich's reagent (p-dimethylaminobenzaldehyde) to give a red chromophore (Bergman and Loxley, 1963).

Woessner (1961) was one of the first to significantly improve the Neuman and Logan (1950) procedure when he developed a method with a recovery rate of one part hydroxyproline from 4000 parts of other amino acids. Bergman and Loxley (1963) made further refinements by optimizing the time and temperature of oxidation and color development, and by optimizing concentrations of organic solvents and reagents at each stage of the procedure. Hence, they were able to achieve greater stability and chromagen sensitivity than had been previously obtained.

Collagen Biosynthesis and Turnover

Collagen molecules are synthesized on membrane bound ribosomes containing mRNA, within the fibroblasts, osteoblasts and chondroblasts (Goldberg et al., 1967; Diegelmann et al., 1973). Seperate mRNA's, which have not been isolated, occur for each of the five α chains, α_1(I), α_1(II), α_1(III), α_1(IV), and α_2 (Miller et al., 1976; Epstein, 1974; Chien, 1975; Martin et al., 1975; Kefalides, 1973). The collagen protein, initially synthesized as the precursor procollagen, contains peptide extensions on both ends of the chain (Tanzer et al., 1974; Davidson et al., 1975; Olsen et al., 1976). Before the chains are secreted from the cell, they are subjected to a series of alterations during and after translation, such as hydroxylation and glycosylation (Dutson, 1976). Both hydroxylation and glycosylation are initiated while the polypeptide chains are growing on the ribosomes and continue after the completed polypeptide chains are released from the ribosomes (Prockop et al., 1976). The three prochains (Figure 2)

of the procollagen molecule are linked by interchain disulfide bonds, occuring at the C terminus, on the completion of synthesis (Byers et al., 1975; Fessler et al., 1975; Bailey and Robins 1976). After helix formation, the procollagen molecule passes through the Golgi apparatus to the cell membrane and into the extracellular matrix. Excess terminal peptides are subsequently removed by the enzyme procollagen peptidase (Figure 2) (Martin et al., 1975; Bailey and Robins, 1976). Once cleavage occurs, the new tropocollagen molecules align themselves into fibers (Dutson, 1976). The fibers then crosslink to stabilize the matrix.

Net accumulation of any protein is dependent on rate of synthesis and degradation (Dayton et al., 1975; Bergen, 1975). Early experiments indicate collagen turnover is very slow (Gerber et al., 1960; Popeno et al., 1962). Recent studies have shown high collagen turnover rates in some tissues, and under some pathological conditions but compared with other proteins, collagen is metabolically inert (Verzar, 1963; Bailey and Robins, 1976).

Dutson (1976) designed an experiment to detect intramuscular collagen turnover in mature ewes. He fed a compound that inhibited the enzyme lysyloxidase, which is necessary for crosslinking of newly synthesized collagen. He detected an increase in the solubility of collagen, indicating that there is intramuscular collagen turnover.

An enzyme, which has been shown to attack native collagen under physiological conditions, was originally isolated from tadpole tissue (Gross et al., 1962) and recently in many other tissues (Harris et al., 1974; Wooley et al., 1975; McCroskery et al., 1975; Wooley et al., 1976). This enzyme has an optimal activity at pH 7.5-8.5, and is activated by calcium ions. The physiological role is unknown, but is believed to be a factor in the natural turnover of collagen in various tissues (Dutson, 1976).

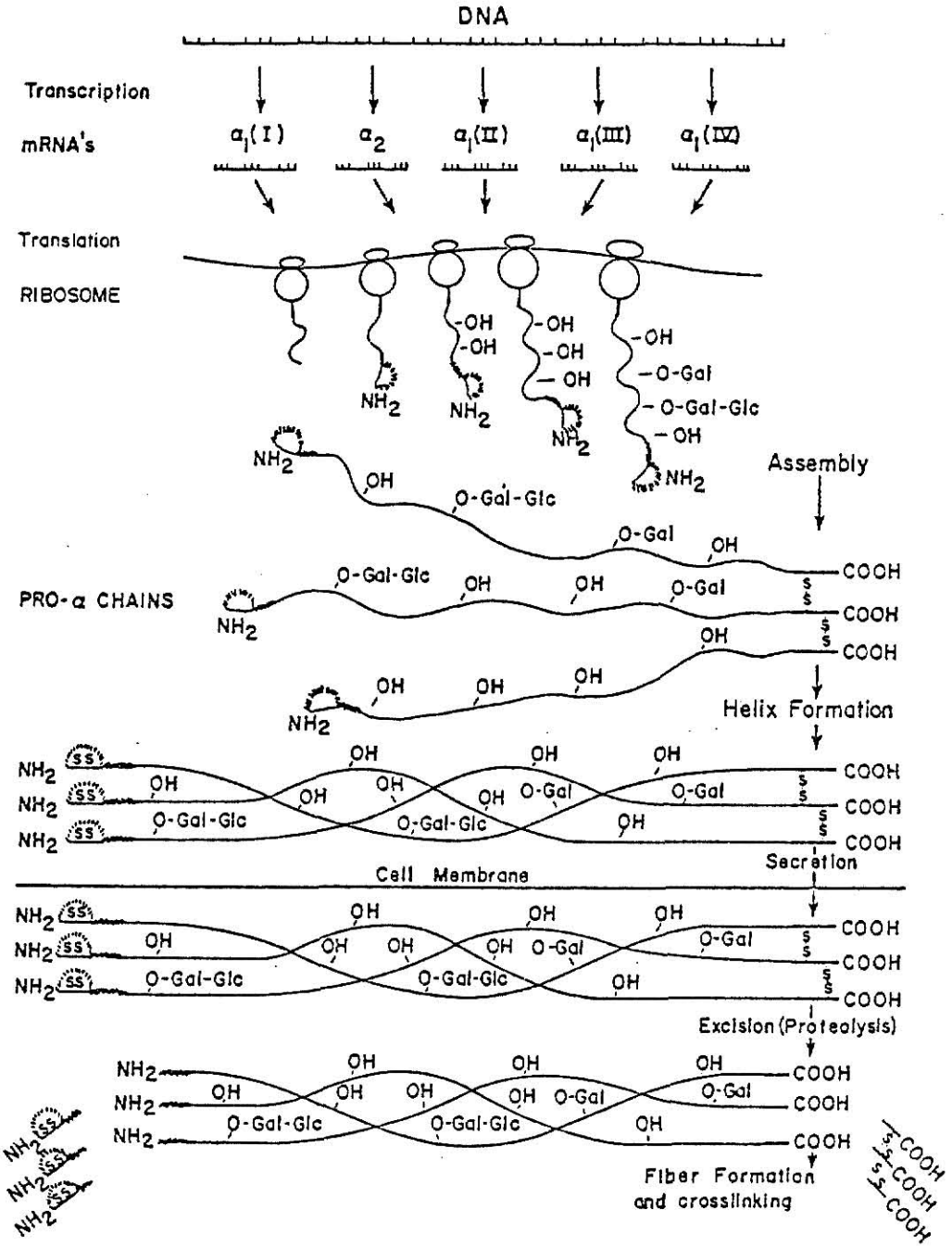

Figure 2. Events involved in collagen biosynthesis (Dutson, 1976).

Connective Tissue Effects on Muscle Tenderness

Tenderness is the major quality factor desired by consumers (Webb et al., 1964). Many factors contribute to variations in beef tenderness and by studying a single component this interrelationship of factors may never be resolved. Kruggel et al., (1970) divided factors affecting beef tenderness into three classifications:

1. antemortem age, genetics, feeding and management practices;
2. postmortem - temperature and storage length after slaughter;
3. structural - molecular properties of the myofibrillar and stromal proteins.

Two structural components, the myofibrillar proteins and, the connective tissue proteins determine the tenderness of meat (Cross et al., 1973; Marsh, 1977). In some circumstances toughness is due almost entirely to connective tissue while in others, it is caused almost exclusively by the contractile proteins (Marsh, 1977). The importance of the contractile proteins on tenderness is acknowledged, but this review will be limited to connective tissue effects on tenderness.

Total Collagen Effects

Studies of the relationship between total muscle collagen and meat tenderness have shown that quantitative changes in total muscle collagen do not occur as tenderness decreases due to increasing market animal age or as tenderness improves with postmortem conditioning (Wierbicki et al., 1954; Goll et al., 1963; McClain et al., 1965; Herring et al., 1967; Berry et al., 1974a; Dutson et al., 1976).

Dutson (1974) adds that tenderness differences between muscles are associated with muscle differences in total collagen. Herring et al. (1967) analyzed the collagen content of two muscles from animals of A, B and E maturities and found no significant differences in total collagen content of the longissimus muscle between maturity groups. However, there was more total collagen ($P<.05$) in the semimembranosus muscle from the E maturity group than muscles from the A or B maturity groups. Data of Field (1968), Herring et al. (1967), Hunsley et al. (1971) and Kruggel et al. (1970) indicate that total collagen in a muscle has no significant effect on tenderness when comparing the same muscle. However, tenderness differences have been attributed to changes within the collagen molecule (Goll et al., 1964c; Kruggel et al., 1970; Nakamura et al., 1975; Shimokomaki et al., 1972). Marsh (1977) summarizes that collagen quantity is of much less significance than its quality.

Goll et al. (1962) and Herring et al. (1967) found that collagen solubility decreased significantly with advancing animal maturity, even though total collagen within muscles was not different. Goll et al. (1964b, c) and Hill (1966) suggested that the number and strength of collagen crosslinkages play an important role in meat tenderness.

Postmortem conditioning causes changes in the molecular structure of collagen. Small molecular weight subunits increase in quantity and the amount of large molecular weight subunits extractable from muscles decrease with postmortem aging (Kruggel and Field, 1971; Pfeiffer et al., 1972; Stanley and Brown, 1973; Dutson, 1974). Stanley and Brown (1973) found that the total amount of extractable collagen increased with postmortem aging. Kruggel and Field (1971) and Pfeiffer et al. (1972) indicate that the amount of extractable low molecular weight collagen

subunits can be increased by stretching a muscle.

McClain (1976) believes collagen undergoes marked changes in crosslinking during postmortem aging. Extractable intramuscular connective tissue rapidly decreased during the first few hours of postmortem aging (McClain et al., 1970); suggesting that the extent of crosslinking increases during this time. McClain et al. (1965) reported a significant increase in crosslinked β components and a decrease in salt-soluble collagen during a 100 hour aging period, indicating that both intra- and inter-molecular crosslinking had occured during aging. These changes apparently influence the ultimate texture and tenderness of meat (McClain, 1976).

Age and Maturity Effects

Wiley and McClain (1976) have shown that from birth to approximately two months of age, there is a marked increase in the yield of soluble collagen from porcine longissimus muscle. Changes in reducible crosslinks in bovine muscle collagen follow this same pattern, but solubility increases up to one year of age (Shimokomaki et al., 1972). These increases reflect the rapid rate of collagen biosynthesis from birth to one year. After twelve months of age, collagen solubility decreases rapidly until about three years of age, then continues to decline slowly throughout the animals life (McClain, 1977). This decrease reflects the conversion of crosslinked intermediates to more stable intermolecular crosslinkages. Thus, as the number of crosslinkages increase, the collagen is less soluble and the meat is less tender (Goll et al., 1967).

Henrickson and Moore (1965) and Shimokomaki et al. (1972) suggest that during the first twelve months of life, rapid bovine muscle growth is due to hypertrophy. This rapid increase in muscle fiber size tends to

"dilute" the connective tissue which contains many reducible crosslinkages. After 12 to 14 months of age, muscle cell hypertrophy slows which seems to trigger the maturation of the connective tissue by increasing the number and strength of highly insoluble intramuscular collagen crosslinks (Goll et al., 1964a; Hill, 1966). The rate of these changes can be affected by an interaction between time-on-feed and animal age (Zinn et al., 1970; Hunsley et al., 1971). Increasing muscle growth during the first 120 to 150 days on feed has a beneficial effect on tenderness but after 180 days on feed (average animal age of 430 days), the maturation of collagen begins to decrease tenderness (Wierbicki et al., 1955; Simone et al., 1958; Webb, 1959; Tuma et al., 1962).

Vognarova et al. (1968) report the greater amount of collagen in veal can be explained by the absolute quantity of connective tissue in the muscle throughout the life of the animal. Only the thickness of connective tissue fibers increase with age by coalescence (Porter, 1951). Muscle fibers, however, continue to increase in diameter and area with age (Hiner et al., 1953). The ratio of connective tissue to total muscle protein decreases with age.

Research studies have failed to identify a specific point in time, chronological age or physiological maturity, at which a definite decrease in tenderness occurs. According to Ho and Ritchey (1967) this point is at about two years of age. They observed significantly lower tenderness scores for meat from animals over two years of age as compared with baby beef. However, Berry et al. (1971, 1972, 1974b) showed that measurements of beef tenderness within physiological maturity are inconsistant, Reagan et al. (1976) found no significant tenderness differences among maturity groups.

Collagen Solubility

The percentage of total collagen is related to tenderness of meat from animals of different ages (Hill, 1966), and is dependent on the extent of intramuscular and intermolecular collagen crosslinkages (Bailey, 1969; Dutson, 1974). Goll et al. (1964c), Hill (1966), Carmichael and Lawrie (1967) and Hunsley et al. (1971) reported a decrease in collagen solubility in nondenaturing solvents as beef tenderness decreased. That portion of collagen soluble in dilute salt solutions at low ionic strengths represents newly synthesized collagen that exists in a loosely-bound form not yet permanently a part of the collagen matrix (Price et al., 1971). Portions soluble in dilute acid solutions represent tropocollagen that is metabolically older, and therefore, has aggregated into collagen fibers and must then be dissociated from this existing tissue (Lowther, 1963). The heat labile fraction requires heating to break down the collagen structure to allow extraction of the immature and weakly crosslinked collagen (Goll et al., 1964b, c; Herring et al., 1971). Mature collagen, the residue remaining after the previous extractions, can be solubilized by enzymatic, physical, or more severe chemical means (Dutson, 1974). To summarize, bovine collagen solubility increases up to a maximum age of 12 to 14 months, then starts to decrease rapidly due to the rapid accumulation of muscle and concomitant formation of labile intermediate collagen crosslinkages. These labile bonds are easily broken by dilute organic acids and together with the noncovalently-bound neutral salt-soluble collagen account for the high solubility (Shimokomaki et al., 1972). The subsequent decrease in solubility after 14 months is due to the decreased rate of collagen synthesis, which allows the labile bonds time to stabilize into nonreducible crosslinkages that render the collagen insoluble.

Reed et al. (1963) states that although solubilization of mature collagen can be achieved, the rate of solubilization does not correspond with the rate of destruction of the bonds. They proposed that as collagen fibrils mature and become insoluble, coatings of mucopolysaccharides and glycoproteins are applied to the fibrils making them more insoluble.

Animal Nutrition Effect on Bovine Collagen

Collagen structure can be modified at certain critical stages in its biosynthesis through some dietary treatments. Inhibition of crosslink formation by use of feed additives, or retardation of intermolecular linkages through controlled nutritional deficiencies are methods of extending the life of the "youthful" form of collagen crosslinkages (Marsh, 1977). Carbohydrates, proteins, certain trace elements and vitamins are recognized nutritional factors having the greatest effect on collagen crosslinking (McClain, 1977). A deficiency in some or all of these factors may lead to decreased collagen crosslinking.

Conventional high concentrate feeding systems actually contribute little to the improvement or assurance of ultimate product quality, causing various alternative feeding systems to be considered in meat production (Anderson et al., 1975; Jeremiah et al., 1971; Smith et al., 1977). Smith et al. (1977) have shown palatibility to be significantly lower ($P<.05$) for steers directly off grass as compared with those finished on high concentrate diets, but these differences disappear when the grass-fed steers were fed high concentrate diets for only 49 days. Prior et al. (1977) suggested that an interaction between diet energy density and protein concentration in the diet affect palatibility. Ferrell et al. (1978) points out that a high energy diet improves rate of gain, a high proportion of which is fat, thus there is very little increase in edible protein.

McClain and Wiley (1975) report that animals starved for a period as short as 24 hours preslaughter may have a reduction of 50-60% in the number of crosslinked components.

Ascorbic acid, iron and vitamin D are important cofactors involved in collagen synthesis. A controlled deficiency of the cofactors may result in intracellular retention of newly synthesized collagen molecules and greater crosslink solubility (Berg and Prockop, 1973). This may result in less collagen available for crosslinking and thus increase overall tenderness (Toole et al., 1972; Barnes et al., 1973; Mechanic et al., 1972).

Copper, another nutritionally derived component, plays a more direct role in collagen crosslinking. Copper deficiency results in a reduction in intermolecular crosslinkages as indicated by a higher proportion of salt- and acid-soluble collagen (Chou et al., 1968). Intramolecular bonding is similarly affected as indicated by the low proportion of β components in salt-soluble collagen (Harris and O'Dell, 1974). Dietary copper affects the activity of lysyl oxidase, an enzyme responsible for the oxidative deamination of lysine residues to the δ-semialdehyde of α-amino adipic acid (Bailey et al., 1974)

Zinc also functions in the process of collagen crosslinking (McClain et al., 1973). Skin collagen is 20% higher in solubility for zinc deficient animals, indicating an inhibition of the crosslinking mechanism or an increase in the number of aldine type crosslinks. Additionally, a 45% reduction in the aldehyde content of skin collagen from zinc deficient animals further suggests inhibition of lysyl oxidase (McClain, 1977). Thus, zinc deficient diets may play a role in producing more tender meat.

Studying the effects of dietary protein on collagen crosslinking,

McClain (1976) observed a significant decrease of about 20% in β components, indicating the presence of more immature collagen in acid-soluble collagen from rats fed a diet restricted to 50% of the normal protein intake. Prasad and Bose (1974) reported a reduction in β components of salt-soluble collagen and a significant decrease in aldehyde content in protein deficient rats, suggesting an impairment of collagen crosslinking. McClain (1977) has also shown that a decrease in collagen biosynthesis can also occur in rats on a limited protein diet. Most importantly, in terms of meat tenderness, it appears that maturation of the reducible crosslinkages to nonreducible-stable crosslinkages can be slowed down if animals are maintained on a low level of dietary protein.

Level and type of dietary carbohydrate also may have an effect on meat tenderness. McClain (1977) suggested that carbohydrate source has a great effect not only on collagen synthesis, but also on the extent and type of intermolecular crosslink formed. Rats fed an all protein diet had similar solubility characteristics as animals on a 65% fructose diet. Rats fed a protein free diet, composed either of 90% glucose or 90% fructose, had lesser amounts of β components compared with rats fed low protein diets. Fructose inhibited crosslinking more than glucose.

The exact mechanism of dietary effects on collagen crosslinking is not totally understood. It is clear that various diets can cause rapid and distinct changes in collagen metabolism and crosslinking. McClain (1977) feels that animal age is not the primary factor affecting collagen's intrinsic capability to form crosslinks, but rather is related to the animals nutritional status.

Cold Shortening Effect on Collagen

Muscle in the pre-rigor state, if not physically restrained, can

contract by 50% or more of its length when exposed to temperature below 10 to 15 C. Locker and Hagyard (1963) found that maximum shortening occurs in unrestrained muscle at 0 C. The ability of a muscle to cold shorten declines with time postmortem, and if the myofibrils are given time enough to complete rigor, the muscle can be cooled below 10 C very rapidly with no appreciable shortening (Marsh, 1977). Locker (1960) was the first to show that muscles with longer sarcomeres were more tender and that the degree of muscle contraction, as permitted by the skeletal attachment, may cause differences in tenderness among muscles rather than muscle differences per se. Hostetler et al. (1972) found sarcomere length to account for 12% of the variation in tenderness among muscles.

For some time after Locker's (1960) discovery of myofibrillar toughness many workers disregarded any effect that connective tissue might have on tenderness due to muscle shortening. Rather, collagen was considered to affect tenderness only by its quantity and animal age relationships. Recent data however, indicate a definite effect of muscle shortening on connective tissue (Bouton and Harris, 1972; Pfeiffer et al., 1972; Rowe, 1974).

Dransfield and Rhodes (1976) found that the arrangement of connective tissue in cold shortened meat definitely contributes to toughness. During muscle shortening tension develops in the helically arranged network of connective tissue. Tension is generated by increases in the cross-sectional area of the collagen matrix as its length is reduced while the volume of collagen remains constant.

Rowe (1974) demonstrated that the sheets of intramuscular connective tissue are made up of a criss-cross lattice of collagen fibers, which at

times are at right angles to the long axis of the muscle fiber. In addition each collagen fiber is crimped (Diamant et al., 1972). Rowe (1974) summarized two changes in the collagen network when muscle shortens. First, as collagen fibers are stretched the crimp length increases or is pulled out. Second, the angles of collagen fibers relative to the long axis of the muscle fiber change with contraction. To study the relationship between tenderness and collagen configuration, Rowe (1974) compared the strength of the sample when the collagen fibers were parallel and perpendicular to the long axis of the muscle fibers. Shortened muscle was toughest, but sample strength of the collagen system was dependent on the number and angle of the collagen fibers. In shortened muscle, the collagen fibers which were parallel to the muscle were shortened and had more crimp. Conversely, collagen fibers that were perpendicular to the shortened muscle were stretched, had less crimp and would have less resistance to shear. Therefore, overall toughness is some permutation of the proportion of parallel and perpendicular collagen fibers relative to the degree of muscle contraction.

Marsh and Leet (1966) and Davey et al. (1967) reported a gradual increase in meat toughness with contraction up to 40% shortening, then a decrease in toughness with over 40% shortening. The crimp of collagen fibers perpendicular to the muscle were gradually pulled out (up to 40% shortening) until in extreme shortening, over 40%, crimp was completely eliminated.

McClain (1976) concluded that connective tissue does play a role in the overall tenderness of a muscle at all sarcomere lengths and it is definitely involved in the increased toughness of cold shortened muscle.

THIS BOOK CONTAINS NUMEROUS PAGES WITH MULTIPLE PENCIL AND/OR PEN MARKS THROUGHOUT THE TEXT.

THIS IS THE BEST IMAGE AVAILABLE.

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Chapter III

COLLAGEN SOLUBILITY OF A-MATURITY BOVINE *LONGISSIMUS* MUSCLE AS AFFECTED BY NUTRITIONAL REGIMEN

Introduction

Variations in beef tenderness have been attributed to the myofibrillar and connective tissue components of muscle (Cross *et al.*, 1973; Marsh, 1977). Connective tissue, mainly collagen, is important in meat tenderness not only because of its abundance in muscle, but also because of its ability to undergo molecular changes during animal maturation (Tanzer, 1973; Cross *et al.*, 1973; Marsh, 1977).

Collagen is generally believed to influence tenderness as an animal increases in chronological age. The myofibrillar component is believed more important in tenderness of meat from young cattle because their collagen lacks extensive crosslinking. This mechanism, however does not sufficiently account for the variations in tenderness of muscles with similar sarcomere lengths from young animals. It is possible that intramuscular connective tissue may be related to these tenderness variations of young A-maturity carcass beef, especially that from cattle fed widely varing nutritional regimens.

Diet energy density may affect molecular changes in collagen and its solubility although the exact mechanism is not well understood (McClain, 1977). McClain (1976) found that protein level and the type and amount of carbohydrate in the diet affected the quantity of reducible collagen crosslinks. Trace elements and vitamins also may affect collagen crosslinking (McClain, 1977).

The purpose of our study was to determine the effect of widely

different nutritional regimens on skeletal muscle collagen solubility from young animals. We were also interested in the relationship between collagen solubility and *longissimus* muscle tenderness.

Materials and Methods

Fifty large type crossbred steers born at the R.L. Hruska US Meat Animal Research Center at Clay Center, Nebraska were randomly assigned in groups of five to one of ten nutritional regimens (Table 3). Steers about 190 days of age were fed corn silage for 90 days before the trial. A control group (treatment 1) of five steers weighing about 300 kg was slaughtered initially. The remaining nine treatment groups were fed either a concentrate or a roughage diet (Table 4). Groups five, six and seven received a concentrate diet fed at maintenance levels or fed ad libitum for 98 or 202 days and then switched to a maintenance level. Feeding periods were terminated at animal end-point (SEP) weights of about 400 kg, 500 kg and 600 kg (SEP-2, SEP-3, SEP-4, respectively). The nutritional regimen required for each group to reach their actual slaughter end-point (SEP) is outlined in Table 3.

Cattle were not fed for 18 to 24 hours before slaughter at either a commercial plant or at the Kansas State University meat laboratory. Carcass data were collected 24 hours postmortem.

Sample Location. Two adjacent steaks each 2.5 cm in thickness were removed seven days postmortem from the 11-12 rib area of the wholesale rib. The anterior steak was used for both taste panel and shear analyses, and the posterior steak was used for both chemical and sarcomere length analyses. Steaks were individually wrapped in freezer paper, frozen and stored at -26 C.

TABLE 3. NUTRITIONAL REGIMENS

Treatment number	Diet	Days fed Ad libitum	Days fed at maintenance	Actual slaughter end-point weight, kg.
1	Control	0	0	298
2	Concentrate	98	0	440
3	Concentrate	202	0	519
4	Concentrate	392	0	630
5	Concentrate-maintenance	0	98	309
6	Concentrate-maintenance	98	104	422
7	Concentrate-maintenance	202	190	446
8	Roughage	126	0	398
9	Roughage	252	0	481
10	Roughage	560	0	666

TABLE 4. DIET COMPONENT PERCENTAGES ON AN AS-FED BASIS

Ingredient	Diet[a]	
	Concentrate	Roughage
Corn (IFN 4-02-931)	61.8	0
Corn silage (IFN 3-08-153)	0	80.5
Alfalfa silage (IFN 3-03-150)	33.1	16.9
Soybean meal (IFN 5-04-604)	4.7	2.6
Supplement[b]	1.0	0.3

[a]Diet energy density, McalME/kg: concentrate = 2.22, roughage = 0.93.

[b]Included limestone, calcium, phosphate and vitamins.

Taste Panel and Shear Force Determinations. Steaks were thawed at 2 C overnight, boned and modified oven broiled (Harrison, 1975) in a rotary gas oven at 163 C to an internal temperature of 70 C as determined by a thermocouple placed in the geometric center of the longissimus muscle.

Six 1.27 cm cores were removed perpendicular to the steak surface with a drill press unit (Kastner and Henrickson, 1969). Cores from five steaks from different treatments were served to a six member trained taste panel per taste panel session according to a 5 x 5 latin square design. A randomly selected block from the latin square was used for the serving order for the sixth panel member. Panel members were instructed to evaluate each sample for connective tissue amount, myofibrillar tenderness, overall tenderness, juiciness and flavor intensity using an eight point scale for each factor (1 = abundant connective tissue, extremely tough, extremely dry or extremely bland flavor; 8 = no detectable connective tissue, extremely tender, extremely juicy or extremely intense flavor). Panelists were first served a "warm-up" longissimus sample (USDA choice) prepared in the same manner as the test steaks. Panelists were screened, trained and tested according to AMSA (1978) recommendations.

After cooling steaks two hours at 25 C, two cores from each of the medial, central and lateral locations of the steak were removed for shear force determination. Each core was sheared in the center with a Warner-Bratzler apparatus and an average shear force was calculated.

Sample Preparation for Collagen Determination. Steaks were thawed at 2 C overnight and all bone, fat and epimysial connective tissue were removed. The steak was cut into strips, frozen in liquid nitrogen and pulverized in a Waring Blendor. The pulverized tissue was placed in a container and stored at -90 C.

Fractionation of Neutral Salt- and Acid-Soluble Collagen. Duplicate 3 g samples of pulverized tissue were weighed into 50 ml centrifuge tubes and salt-soluble collagen was extracted according to Fielding (1976, Appendix A). The supernatant was prepared for salt-solubilized hydroxyproline determination. The residue was used for extraction of acid-soluble collagen according to a modification of the procedure of Bazin and Delaunay (1976, Appendix B). The modification included use of .5 M acetic acid, an extraction time of 48 hours and centrifugation at 18,000 rpm for 1 hour. This supernatant was prepared for acid-solubilized hydroxyproline determination.

Preparation of Salt- and Acid-Solubilized Fractions for Hydroxyproline Determination. Three ml of supernatant from either the salt or acid fraction was mixed with an equal volume of concentrated HCl and autoclaved for 6 hours at 121 C. Contents of the tubes were dried by a stream of moving air in a 70 C water bath and 1 ml deionized water was added, swirled and redried. After cooling, 3 ml of deionized water was added with 50 mg of charcoal to clarify the solution. The samples were filtered (Whatman #1) and duplicate 1 ml portions of the filtrate were used for hydroxyproline determination.

Total Collagen. A modified procedure of Stegemann and Stadler (1967, Appendix D) was used for total collagen quantitation. Modifications consisted of neutralization of the cool autoclaved sample with saturated LiOH, bringing the volume to 250 ml with deionized water, adding charcoal and filtering. The filtrate was prepared for hydroxyproline determination.

Hydroxyproline Determination. Hydroxyproline was determined by a modification of Bergman and Loxley (1963, Appendix E). This modification

involved the addition of 2 ml Ehrlich's reagent, heating in water bath as described and then cooling in running tap water for 5 minutes and measurement of absorbance within 30 minutes without dilution.

Sarcomere Length. Frozen pulverized tissue (.5 to .7 g) was blended in 300 ml of cold .25 M sucrose for 30 seconds at a medium speed in a Virtis mixer. One drop of the mixture was placed on a slide, and sarcomere lengths were measured with a Wild phase contrast microscope at 750 X. Total length of 10 sarcomeres on each of 20 myofibrils were measured with an eyepiece filar micrometer to estimate average sarcomere length.

Statistical Analysis. A balanced incomplete block design was used to assign taste panel steaks to serving days. An additional treatment of steaks was added to balance the design; but this treatment was disregarded in the final analysis.

Analysis of variance with resultant F-test was used in analyzing all data. Least significant difference values were used to seperate means. Simple correlation coefficients were determined between selected traits.

Results and Discussion

Characterization of Carcasses. Chronological age of the steers ranged from 280 to 840 days and their carcass physiological maturity was either A-minus or A-typical (Table 5). Quality grades ranged from Standard to Choice and were related to dietary energy level. Carcasses from cattle fed concentrate ad libitum had higher quality grades at slaughter end point two (SEP-2) than carcasses from cattle fed roughage ad libitum. However, both groups graded Good at SEP-3 and Choice at SEP-4. Carcasses from the concentrate-maintenance (CM) groups had the lowest quality grades at SEP-2, SEP-3 and SEP-4. Yield grades were not different ($P>.05$) between diet groups through SEP-3 except that carcasses from the concentrate group had higher ($P<.05$) yield grades than those from the roughage fed group at SEP-2. Yield grades from the restricted energy intake group (CM) were lowest at SEP-2 and SEP-4. Actual fat thickness (Table 5) was greatest for carcasses from the concentrate fed groups, intermediate for the roughage groups and lowest for carcasses from the CM groups. Thus, based on fat thickness, if cold shortening and its subsequent effect on collagen crosslinking (Pfeiffer et al., 1972; Rowe, 1974) and muscle toughening were to occur, the control group and CM groups at SEP-2 to SEP-4 and the roughage groups at SEP-2 and SEP-3 would be most susceptible.

Diet Effects on Total Collagen. Total longissimus intramuscular collagen content (Table 6) from cattle at SEP-1 and SEP-2 were similar ($P>.05$). Muscle from cattle fed the concentrate diet at SEP-3 had less collagen than the muscle from cattle fed roughage at SEP-3. Cattle fed

TABLE 5. MEANS FOR CARCASS MATURITY, USDA QUALITY AND YIELD GRADES AND ACTUAL FAT THICKNESS FOR CATTLE FED DIFFERENT NUTRITIONAL REGIMENS

Diet[b]	Slaughter end-point[a] 1	2	3	4
		Average maturity[c]		
Control	A-^{e}			
Concentrate		A-^{e}	A-^{e}	Ao^{f}
Concentrate-maintenance		A-^{e}	A-^{e}	A-^{e}
Roughage		A-^{e}	A-^{e}	Ao^{f}
		USDA quality grade[cd]		
Control	So^{hi}			
Concentrate		G+$^{f}_{i}$	G-$^{fg}_{hi}$	Co^{e}
Concentrate-maintenance		S-^{i}	So^{f}	Go^{f}
Roughage		S+^{gh}	Go^{f}	Co^{e}
		USDA yield grade		
Control	1.4^{gh}			
Concentrate		2.1^{fg}	1.7^{gh}	3.6^{e}
Concentrate-maintenance		1.3^{gh}	1.7^{gh}	1.7^{gh}
Roughage		1.0^{h}	1.7^{gh}	2.8^{ef}
		Actual fat thickness,cm		
Control	0.3^{hi}			
Concentrate		0.6$^{g}_{i}$	0.7$^{fg}_{h}$	1.4$^{e}_{hi}$
Concentrate-maintenance		0.1^{hi}	0.4^{h}	0.3^{f}
Roughage		0.3^{hi}	0.4^{h}	0.9^{f}

[a]Approximate slaughter end-point animal weights of 300, 400, 500 and 600 kg respectively, see Table 3.

[b]Control=cattle killed at start of trial; Concentrate=cattle fed concentrate diet ad libitum; Concentrate-maintenance=cattle fed concentrate diet ad libitum then restricted to maintenance intake when previous end-point weight was obtained; Roughage=cattle fed roughage diet ad libitum.

[c]Maturity or grade ranges: minus=0 to 33%, typical (o)=34 to 66%, plus=67 to 100%.

[d]S=Standard; G=Good; C=Choice.

[e,f,g,h,i]Means with same superscript letter for each trait are not different ($P > .05$).

TABLE 6. MEANS FOR LONGISSIMUS MUSCLE COLLAGEN CHARACTERISTICS FOR CATTLE FED DIFFERENT NUTRITIONAL REGIMENS

Diet[b]	Slaughter end-point[a] 1	2	3	4
		Total collagen, mg/g fresh tissue		
Control	4.22^{de}			
Concentrate		4.32^{de}	3.74^{e}	4.19^{de}
Concentrate-maintenance		4.64^{d}	3.96^{de}	5.71^{c}
Roughage		4.22^{de}	4.78^{d}	4.34^{de}
		Salt-soluble collagen, percent of total collagen		
Control	2.37^{cde}			
Concentrate		1.96^{def}	2.29^{cde}	1.86^{def}
Concentrate-maintenance		1.74^{ef}	2.55^{cd}	1.36^{f}
Roughage		1.68^{ef}	1.81^{def}	3.05^{c}
		Acid-soluble collagen, percent of total collagen		
Control	3.54^{cd}			
Concentrate		3.02^{cd}	3.44^{cd}	2.83^{cd}
Concentrate-maintenance		2.92^{cd}	3.23^{cd}	2.51^{d}
Roughage		3.78^{c}	2.98^{cd}	3.10^{cd}
		Salt- plus acid-soluble collagen, percent of total collagen		
Control	5.91^{cd}			
Concentrate		4.98^{cdef}	5.72^{cde}	4.68^{def}
Concentrate-maintenance		4.66^{ef}	5.78^{cde}	3.87^{f}
Roughage		5.47^{cde}	4.78^{def}	6.15^{c}

[a]Approximate slaughter end-point animal weights of 300, 400, 500 and 600 kg respectively, see Table 3.

[b]Control=cattle killed at start of trial; Concentrate=cattle fed concentrate diet *ad libitum*; Concentrate-maintenance= cattle fed concentrate diet *ad libitum* then restricted to maintenance intake when previous end-point weight was obtained; Roughage=cattle fed roughage diet *ad libitum*.

[c,d,e,f]Means with same superscript letter for each trait are not different (P>.05).

the CM diet to SEP-4 had more ($P<.05$) total collagen in the longissimus muscle than any other group.

In general, diet effects on total collagen were small and no consistant trend was evident; however, the following is speculated. Slight decreases in total collagen from SEP-2 to SEP-3 in longissimus muscle from cattle fed the concentrate and CM diets may be attributed to a dilution effect (Carmichael and Lawrie, 1967) due to rapid myofibrillar growth. But by SEP-4, the proportion of total collagen was higher possibly due to the decreased rate of myofibrillar growth. Similar changes in collagen tended to occur in the roughage groups, but the dilution of collagen did not occur until SEP-4 because of the slower initial growth rate of animals fed silage.

Total collagen data generally agree with those of Corte (1977) who found significantly more longissimus muscle collagen in cattle fed on lower planes of nutrition. Wu et al. (1981), however, did not find significant differences in longissimus collagen content between grass-fed and concentrate-fed cattle.

Diet Effects on Salt- and Salt- plus Acid-Soluble Collagen. Salt-soluble collagen represents the most recently synthesized collagen (Price et al., 1971), and collagen soluble in dilute acids is metabolically older tropocollagen that has aggregated into collagen fibers (Lowther, 1963).

Diet effects on salt-soluble and salt- plus acid-soluble collagen did not vary consistantly (Table 6). No differences ($P>.05$) in solubility due to diet occured through SEP-3 except for an increase in the CM group's

salt-soluble collagen from SEP-2 to SEP-3. Steaks from the CM group at SEP-3 had more ($P<.05$) salt-soluble collagen than the CM groups at SEP-2 and SEP-4 or the roughage group at SEP-2. At SEP-2, steaks from the CM group had less ($P<.05$) salt- plus acid-soluble collagen than the control group and the roughage group at SEP-4. At SEP-4, steaks from the CM group had the lowest and the roughage group the highest percentages of salt-soluble and salt- plus acid-soluble collagen.

Changes in collagen solubility at SEP-4 for all nutritional regimens suggests interrelationships between diet and animal age. Animals fed the high energy diets had a high percentage of soluble collagen during their rapid growth phase. But, since they reached a more mature weight earlier than animals fed lower energy diets, their connective tissue became less soluble sooner. This effect was most notable in the CM group fed 202 days and switched to a maintenance diet for 190 days (SEP-4).

Apparently, the collagen is more subject to crosslinking in muscle of cattle fed high energy diets and subsequently switched to diets restricting energy intake. Conversely, cattle fed roughage diets may grow slower and reach a mature weight at an older age. Consequently, their collagen solubilities do not decrease until they are chronologically older than concentrate-fed cattle.

Diet Effect on Acid-Soluble Collagen. Acid-soluble collagen from the longissimus muscle was not affected ($P>.05$) by diet through SEP-3. At SEP-4 the CM group had less acid-soluble collagen ($P<.05$) than the roughage group at SEP-2. Corte (1977) also found diet had little effect on acid-soluble collagen.

Diet Effects on Palatability Traits and Warner-Bratzler Shear Values. Diet had no major effects on palatability traits and Warner-Bratzler shear values through SEP-3 (Table 7). In general, scores for myofibrillar tenderness, connective tissue amount and overall tenderness were lowest and Warner-Bratzler shear values were highest for the concentrate fed group at SEP-4. Values for these traits were intermediate for the CM groups at SEP-4, and palatability scores were highest and Warner-Bratzler shear values lowest for steaks from the group fed roughage compared to other groups at SEP-4. Tenderness scores decreased and the amount of detectable connective tissue and Warner-Bratzler shear values increased for the concentrate and CM groups from SEP-2 to SEP-4. Although not significant, tenderness and shear values improved slightly for steaks from the roughage groups from SEP-2 to SEP-3; however, steaks from these groups became less tender from SEP-3 to SEP-4.

Our data agrees with Simone et al. (1968), Webb (1959), Tuma et al. (1962) and Zinn et al. (1970) who reported that feeding cattle concentrate diets more than 180 to 210 days (SEP-3 in this study) is detrimental to longissimus muscle tenderness. McClain (1977) found animals on a limited protein diet had decreased collagen biosynthesis and less conversion of reducible crosslinkages to non-reducible, stable crosslinkages. Our roughage fed cattle had characteristics similar to animals on limited proteins diets even though the digestable protein per unit of metabolizable energy was equal in all our diets. Perhaps protein source also influences collagen biosynthesis and crosslinking. Further, our results agree with Bayne et al. (1969), Bidner (1975), Smith et al. (1979), Burson et al. (1980) and Wu et al. (1981) that diet has little influence on palatability

TABLE 7. LONGISSIMUS MUSCLE TASTE PANEL, WARNER-BRATZLER SHEAR FORCE AND SARCOMERE LENGTH MEANS FOR CATTLE FED DIFFERENT NUTRITIONAL REGIMENS

	Slaughter end-point[a]			
Diet[b]	1	2	3	4
		Myofibrillar tenderness[c]		
Control	6.1^{de}			
Concentrate		6.5^{d}	6.3^{d}	5.0^{e}
Concentrate-maintenance		6.5^{d}	6.1^{de}	5.6^{de}
Roughage		6.2^{d}	6.5^{d}	6.0^{de}
		Connective tissue amount[c]		
Control	7.0^{d}			
Concentrate		7.2^{d}	7.0^{d}	6.0^{e}
Concentrate-maintenance		7.2^{d}	6.7^{de}	6.2^{e}
Roughage		7.1^{d}	7.1^{d}	6.8^{de}
		Overall tenderness[c]		
Control	6.3^{de}			
Concentrate		6.6^{d}	6.5^{d}	5.1^{f}
Concentrate-maintenance		6.6^{d}	6.1^{def}	5.3^{ef}
Roughage		6.4^{d}	6.7^{d}	6.1^{def}
		Warner-Bratzler shear force, kg		
Control	3.7^{efg}			
Concentrate		3.3^{fg}	3.9^{efg}	5.3^{d}
Concentrate-maintenance		2.8^{g}	4.1^{def}	4.6^{de}
Roughage		3.4^{fg}	3.1^{fg}	3.9^{efg}
		Sarcomere length; μm		
Control	1.73^{e}			
Concentrate		1.92^{d}	1.85^{de}	1.90^{de}
Concentrate-maintenance		1.77^{e}	1.77^{e}	1.82^{de}
Roughage		1.96^{d}	1.93^{d}	1.85^{de}

[a]Approximate slaughter end-point animal weights of 300, 400, 500 and 600 kg respectively, see Table 3.

[b]Control=cattle killed at start of trial; Concentrate=cattle fed concentrate diet *ad libitum*; Concentrate-maintenance=cattle fed concentrate diet *ad libitum* then restricted to maintenance intake when previous end-point weight was obtained; Roughage=cattle fed roughage diet *ad libitum*.

[c]Scores based on an eight point scale for each trait (1=abundant connective tissue or extremely tough; 8=no connective tissue or extremely tender).

[d,e,f,g]Means with same superscript letter for each trait are not different (P>.05).

characteristics or shear force values of beef if fed to comparable end-points. My results suggest, as did those of Burson et al. (1980), that if time on feed affects beef palatability the effect may occur before the start of the trial either during the growing period or the pretrial adjustment (Table 8) period.

Myofibrillar tenderness had higher correlations with overall tenderness and Warner-Bratzler shear values (r=.95 and -.72 respectively) than did connective tissue amount (r=.88 and -.66 respectively). Therefore, tenderness differences in this study may have been due more to myofibrillar effects than to connective tissue factors. Cold shortening was probably not a factor affecting tenderness since steaks which were least tender came from cattle which generally had more fat thickness than cattle which produced steaks rated highest for tenderness. Correlations ($P<.05$) for total collagen with myofibrillar tenderness, connective tissue amount and overall tenderness were -.30, -.44 and -.39, respectively (Table 8). Correlations between measures of collagen solubility and palatability traits were low ($r<.26$) and non-significant (Table 8).

Diet Effects on Sarcomere Length. Longissimus muscle sarcomere length (Table 7) tended to be longer for nutritional regimens where cattle were gaining weight and were fatter (Table 5). Sarcomere length for cattle in the control and CM groups were shortest, perhaps the result of cold shortening. This shortening of sarcomeres may cause simultaneous structural changes in collagen that tend to increase resistance to shear (Dransfield and Rhodes, 1976; Rowe, 1974) and decrease collagen solubility (Pfeiffer et al., 1972). Conversely, Rhodes and Dransfield, (1974) report that the connective tissue contribution to shear value in raw tissue is less in shortened muscle than in stretched muscle. However,

TABLE 8. CORRELATION COEFFICIENTS FOR COLLAGEN CHARACTERISTICS, TASTE PANEL TRAITS, WARNER-BRATZLER SHEAR VALUES, SARCOMERE LENGTH AND CARCASS FAT THICKNESS

Parameter	TC	SS	AS	MT	CT	OT	WB	SL
Total collagen (TC)	1.00							
Salt-soluble collagen (SS)	-0.43[a]							
Acid-soluble collagen (AS)	-0.26	-0.06						
Myofibrillar tenderness (MT)	-0.30[b]	0.25	0.08					
Connective tissue amount (CT)	-0.44[a]	0.17	0.10	0.74[a]				
Overall tenderness (OT)	-0.39[a]	0.25	0.12	0.95[a]	0.88[a]			
Warner-Bratzler shear (WB)	0.13	-0.04	-0.11	-0.72[a]	-0.66[a]	-0.76[a]		
Sarcomere length (SL)	-0.17	0.03	-0.01	0.41[a]	0.36[b]	0.39[a]	-0.37[a]	
Fat thickness (FT)	-0.30[b]	0.29[b]	-0.03	-0.15	-0.13	-0.13	0.31[b]	0.17

[a] $P<.01$.

[b] $P<.05$.

sarcomere length was not strongly correlated (Table 8) with measures of tenderness ($r<.42$), actual fat thickness ($r=.17$), total collagen ($r=-.17$) and measures of collagen solubility ($r<.04$).

Collagen Solubility and Tenderness. Since palatability differences (Table 7) between nutritional regimens were small, I investigated the relationship between collagen solubility and tenderness by stratifying our data into two tenderness groups. Overall tenderness scores were used to establish two groups consisting of the 15 least tender and 16 most tender longissimus muscles (Table 9). Myofibrillar tenderness, connective tissue amount, overall tenderness and Warner-Bratzler shear values were significantly different ($P<.05$) for the two groups. Collagen solubility did not differ ($P>.05$) between the two tenderness groups; but, there was a trend for the most tender group to have less total collagen ($P<.10$), and more salt-soluble collagen ($P<.14$). Acid-soluble collagen was not different for these two tenderness groups.

The primary objective of this study was to relate collagen solubility to nutritional regimen. Cattle varied widely in feeding history and age within the A-maturity group; however, no definite relationship between collagen solubility and nutritional regimen was established. Trends indicated that high diet energy density may be related to initiation of collagen crosslinking, especially when energy is limited after ad libitum feeding.

We conclude that nutritional regimen had little effect on collagen solubility as measured in this study; and that soluble collagen characteristics are not likely to account for much of the tenderness variation in longissimus muscle from A-maturity carcasses.

TABLE 9. LEAST SQUARE MEANS FOR TASTE PANEL TENDERNESS, WARNER-BRATZLER SHEAR FORCE AND SOLUBLE COLLAGEN OF BOVINE LONGISSIMUS MUSCLE BY TENDERNESS GROUP[a]

Variable	Tenderness Group		Probability
	Most Tender	Least Tender	
Myofibrillar tenderness[b]	6.9	5.3	0.0001
Connective tissue amount[b]	7.4	6.3	0.0001
Overall tenderness[b]	7.0	5.3	0.0001
Warner-Bratzler shear force, kg.	3.0	4.6	0.0001
Total Collagen, mg/g	4.28	4.77	0.094
Salt-soluble collagen, %	2.08	1.72	0.134
Acid-soluble collagen, %	3.14	3.15	0.987
Salt+acid-soluble collagen, %	5.22	4.86	0.388

[a]Groups stratified as most tender (n=16) and least tender (n=15) based on overall tenderness scores.

[b]Scores based on an eight point scale for each trait (1=abundant connective tissue or extremely tough; 8=no connective tissue or extremely tender).

Summary

Fifty large type crossbred steers were assigned in groups of five to one of ten nutritional regimens to measure the effects of diet on collagen characteristics. A control group was slaughtered initially at about 280 days of age and about 300 kg. The remaining 9 treatment groups, were fed either roughage ad libitum, concentrate ad libitum or concentrate at a maintenance (CM) level. Animal slaughter end-point (SEP) weights were about 400 kg (SEP-2), 500 kg (SEP-3) and 600 kg (SEP-4). Feeding times ranged from 0 to 560 days. Carcasses were all A-maturity and the USDA quality grades ranged from low Standard to average Choice. Quality grades generally were highest for carcasses from the concentrate group, intermediate for the roughage group and lowest for the CM group. Diet effects on total collagen were small and inconsistent. Acid-soluble collagen was not affected ($P>.05$) by diet. Differences in percentages of salt-soluble and salt- plus acid-soluble collagen were small through SEP-3. At SEP-4, the percentages of salt-soluble and salt- plus acid-soluble collagen were lower ($P<.05$) in steaks from the concentrate and CM groups than in steaks from the roughage group. Variations in longissimus sarcomere length did not effect collagen solubility ($r<.03$). Diet had no effect on palatability traits or Warner-Bratzler shear (WBS) values through SEP-3. At SEP-4, palatability scores were lowest and WBS values the highest for the concentrate group, intermediate for the CM group and the most desirable for the roughage group. Due to

small palatability differences between nutritional regimens, data were stratified into tender (n=16) and less tender (n=15) groups based on overall tenderness. These two groups were different ($P<.05$) for myofibrillar tenderness, connective tissue amount, overall tenderness and Warner-Bratzler shear values; but, measures of collagen solubility were not different ($P>.05$) between the two groups. The tender group tended to have less total collagen ($P<.10$) and more salt-soluble collagen ($P<.14$). Acid-soluble collagen was not different between the two tenderness groups.

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APPENDICES

APPENDIX A

FRACTIONATION OF NEUTRAL SALT-SOLUBLE COLLAGEN[a]

1. Weigh duplicate 3 g samples into a 50 ml centrifuge tube. Add 10 volumes (30 ml) buffered 0.5 M NaCl solution (pH 7.4). Extract samples with shaking for 24 hr at 2-4 C.

2. Remove tubes from shaker, balance, and centrifuge at 18,000 RPM for 30 min at 5 C. Decant supernatant and analyze it for salt-soluble hydroxyproline.

3. Rinse the residue with 10 volumes (30 ml) deionized H_2O, stir, centrifuge at 18,000 RPM for 30 min. Decant and discard supernatant. Analyze residue for acid-soluble collagen content.

APPENDIX B

FRACTIONATION OF ACID-SOLUBLE COLLAGEN[b]

1. Extract residue from the salt-soluble determination in 50 ml centrifuge tubes by shaking for 48 hrs at 2-4 C in 10 volumes (30 ml) of 0.5 M acetic acid.

2. Remove from shaker, balance and centrifuge at 18,000 RPM for 1 hr at 5 C.

3. Decant supernatant and analyze it for acid-soluble hydroxyproline. The residue can be discarded or analyzed for residual hydroxyproline.

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[a]Fielding, A.M. 1976. Preparation of neutral salt-soluble collagen. In D.A. Hall (Ed.). The Methodology of Connective Tissue Research, 1st Edition. Joynson-Bruvvers Ltd., Oxford England. p. 9.

[b]Bazin, S. and A. Delaunay 1976. Preparation of acid and citrate soluble collagen. In D.A. Hall (Ed.). The Methodology of Connective Tissue Research, 1st Edition. Joynson-Bruvvers Ltd., Oxford, England. p. 13.

APPENDIX C

SALT- AND ACID-SOLUBLE HYDROXYPROLINE PREPARATION

1. Take 3 ml of supernatant from either the salt or acid fractions, add 3 ml (equal volume) concentrated HCl and place in either:

   a) a large (50 ml) screw top test tube (cover loosely) if aspiration is used for drying.
   b) a 25 ml Erlenmeyer flask, covered with large marble if drying is with a stream of moving air.

2. Autoclave at least 6 hours or overnight, at 16-19# of pressure (122-216 C).

3. Cool autoclaved sample to room temperature.

4. Dry the sample by either aspiration or moving air in a 70 C water (shaker bath) bath until dry. Add 1 ml deionized H_2O, swirl and redry.

5. When dry, remove from bath, cool, and add a volume equal to the size of sample (3 ml) of deionized H_2O, swirl.

6. Add small amount (50 mg) of charcoal to clarify.

7. Filter samples into a small test tube (10 - 15 ml) through Whatman #1 filter paper cut to fit a 25 mm filtering funnel.

8. Take duplicate 1 ml portions of the filtrate and analyze for hydroxyproline using either the rapid or overnight procedures (modified) of Bergman and Loxley.

THIS BOOK CONTAINS NUMEROUS PAGES THAT HAVE INK SPLOTCHES IN THE MIDDLE OF THE TEXT. THIS IS AS RECEIVED FROM CUSTOMER.

THESE ARE THE BEST IMAGES AVAILABLE.

APPENDIX D

DETERMINATION OF TOTAL COLLAGEN[a]

1. Weigh duplicate 3 g samples of pulverized muscle into a 250 ml Erlenmeyer, or 50 ml seren top test tube and add 10 volumes (30 ml) of 6.0 N HCl. Cover loosely.

2. Autoclave for at least 6 hours or overnight at 16-19# pressure (122-216 C).

3. Cool autoclaved sample to room temperature.

4. Neutralize, with saturated LiOH using a volume approximately equal that of the added HCl (30 ml). Test for neutrality with phenolphthalein and adjust pH to 6-7 with dilute acetic acid.

5. Bring volume to 250 ml in a volumetric flask (or 250 ml graduated cylinder) with deionized H_2O.

6. Add charcoal to clarify (350-400 mg).

7. Filter samples through Whatman #1 filter paper into a 400 ml beaker.

8. Mix and analyze duplicated 1 ml aliquots of filtrate for hydroxyproline content using either the rapid or overnight procedures (modified) of Bergman and Loxley.

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[a] Stegemann, H. and K. Stadler 1967. Determination of hydroxyproline. Clinica Chimica Acta 18:267.

APPENDIX E

DETERMINATION OF HYDROXYPROLINE BY A MODIFIED BERGMAN AND LOXLEY PROCEDURE

Rapid Procedure

1. Pipette 1 ml aliquots of the neutral or slightly acid solution from the extraction procedures into clean, dry 30 ml glass test tubes.
2. Add (pipette) 2 ml of isopropanol and mix.
3. Add (pipette) 1 ml of oxidant solution, mix well and allow to stand 4 (±1) min at room temperature (17-21 C).
4. Add (pipette) 2 ml Ehrlich's reagent and mix well.
5. Heat tubes for 25 min (±15 sec) at 60 C (±0.2 C) in a water bath. Be sure tubes are covered to limit evaporation.
6. Cool the tubes for 5 min in running tap water.
7. Shake the tubes after cooling just before reading.
8. Measure absorbance at 558 nm against a 0 µg/ml standard as soon as possible (½ hour maximum) in a 1 cm cuvette.
9. Results, expressed as micrograms of hydroxyproline per gram of wet tissue. To convert to micrograms of collagen per gram of wet tissue multiply by .00725.

Note:

1. A reagent blank was included by substituting deionized water (0 µg/ml standard) for the unknown solution and the absorbances corrected accordingly. Whenever a new batch of any of the solvents or reagents is used, 4-7 hydroxyproline standard solutions between 5-40 ppm were included.

Reagent Formulation for Rapid Procedure:

Oxidant Solution:

A. 0.35 g chloramine T dissolved in 5 ml deionized water (pH 7.5).

B. Acetate/citrate Buffer:
57 grams Sodium Acetate - 3 H_2O or 34.4 grams Sodium Acetate anhydrous
37.5 grams Trisodium citrate - 2 H_2O

5.5 grams citric acid - 1 H_2O
385 ml isopropanol
Combine above ingredients and dilute up to 1 liter with deionized water.
Oxidant Solution: Mix 1 volume of A to 4 volumes of B. Should be made fresh, within 3-4 hours before use.

Note:

1. If chloramine T is insoluble in the water, it is no good or, if the samples after color development are turbid the chloramine T may be partially inactive.

2. The acetate/citrate buffer as formulated above will be about pH 6.5. As stated in some literature the buffer should be pH 6.0. This adjustment can be made by adjusting the pH to 6.0 with dilute acetic acid before the addition of the alcohol. This is necessary because alcohols have no pH and will cause an erroneous pH if the buffer is tested after the isopropanol has been added. However, the presence of the acetic acid in the buffer may cause turbidity when added to the chloramine T, so it is recommended to leave the buffer unadjusted as long as it is close to pH 6.5.

3. Since there is a large quantity of alcohol in the solution, the growth of microorganisms is minimal. It can therefore be stored at room temperature. If stored in a refrigerator, crystalization will occur and the buffering capacity is lost. To reactivate, the solution must be warmed until everything is back in solution. Make fresh buffer every 2-3 weeks to insure activity.

Ehrlich's Reagent.

A. 2 grams p-Dimethylaminobenzaldehyde (DABA) dissolved in 2 ml of 60% or 2.5 ml of 70% perchloric acid.

B. Isopropanol

Ehrlich's reagent: mix 3 volumes of A with 13 volumes of B.

Note:

1. Solution A can be made in a quantity of 1 liter or more and stored about 4 weeks in a brown bottle.

2. Final color development of various shades of green instead of pink and red indicates inactive Ehrlich's reagent.

Modification of the Bergman and Loxley rapid procedure is only in that 2 ml of Ehrlich's reagent is used rather than 13 ml as recommended in that paper.

Overnight Procedure:

This procedure does not require dilution to stabilize the final

color; it is suitable for the analysis of solutions containing 2-15 ppm hydroxyproline (ideally 6-12 ppm). Procedure is conveniently carried out in glass-stoppered tubes, with color development at room temperature. The overnight and the rapid procedures differ only in the preparation of Ehrlich's reagent. For the overnight procedure, the Ehrlich's reagent is made as follows: The aldehyde (DABA) is dissolved in 60% perchloric acid. Just before the start of each series of determinations, this solution is mixed with isopropanol in the proportions of 3 ml to 16 ml of isopropanol. Absorbance is measured at 558 nm after about 17 hours. This procedure is from Bergman and Loxley, Clinica Chimica Acta. 1970. 27:347.

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[a] Bergman, I. and R. Loxley. 1963. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. Anal. Chem. 35:1961.

APPENDIX F

PALATABILITY SCORE SHEET

Panelist's Name: _____ Date: _____

Sample	Muscle Fiber Tenderness	Amount of Connective Tissue	Overall Tenderness	Juiciness	Flavor Intensity
1					
2					
3					
4					
5					
6					
7					

Muscle Fiber Tenderness	Amount of Connective Tissue	Overall Tenderness	Juiciness	Flavor Intensity
8. Extremely Tender	8. None	8. Extremely Tender	8. Extremely Juicy	8. Extremely Intense
7. Very Tender	7. Practically None	7. Very Tender	7. Very Juicy	7. Very Intense
6. Moderately Tender	6. Traces	6. Moderately Tender	6. Moderately Juicy	6. Moderately Intense
5. Slightly Tender	5. Slight	5. Slightly Tender	5. Slightly Juicy	5. Slightly Intense
4. Slightly Tough	4. Moderate	4. Slightly Tough	4. Slightly Dry	4. Slightly Bland
3. Moderately Tough	3. Slightly Abundant	3. Moderately Tough	3. Moderately Dry	3. Moderately Bland
2. Very Tough	2. Moderately Abundant	2. Very Tough	2. Very Dry	2. Very Bland
1. Extremely Tough	1. Abundant	1. Extremely Tough	1. Extremely Dry	1. Extremely Bland

COLLAGEN SOLUBILITY OF A-MATURITY BOVINE *LONGISSIMUS* MUSCLE AS AFFECTED BY NUTRITIONAL REGIMEN

by

JAMES BRIAN HALL

B.S., Kansas State University, 1978

AN ABSTRACT OF A MASTER'S THESIS

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The purpose of this study was to measure the effect of nutritional regimen on collagen solubility and maturation and their relationship to A-maturity bovine longissimus muscle tenderness.

Fifty large type crossbred steers were randomly assigned in groups of five to one of ten nutritional regimens after a 90 day adjustment period on a corn silage diet. A control group of 5 steers (treatment 1) was slaughtered initially (Slaughter end-point 1) at about 280 days of age and about 300 kg. Of the remaining 9 treatment groups, 3 were fed roughage ad libitum, 3 were fed concentrate ad libitum and 3 were fed a concentrate diet at a maintenance (CM) level or fed concentrate ad libitum for either 98 or 202 days and then switched to concentrate at a maintenance level. Feeding periods were terminated at animal slaughter end-point (SEP) weights of about 400 kg, 500 kg and 600 kg (SEP-2, SEP-3, SEP-4, respectively). Feeding times ranged from 0 to 560 days. Carcass data were collected at 24 hours postmortem. Carcasses were all A-maturity and the USDA quality grades ranged from low Standard to average Choice. Quality grades generally were highest for carcasses from the concentrate group, intermediate for the roughage group and lowest for the CM group. Longissimus steaks were removed seven days postmortem for collagen, taste panel, shear force and sarcomere length analysis.

Diet effects on total collagen were small. Total collagen in steaks from the concentrate and CM groups tended to decrease from SEP-2 to SEP-3, but increased slightly by SEP-4. Steaks from the roughage fed group increased in total collagen through SEP-3 and then decreased at SEP-4.

Diet had no effect ($P>.05$) on acid-soluble collagen.

Differences in percentage of salt-soluble and salt- plus acid-soluble collagen were relatively small through SEP-3. At SEP-4, the percentages

of salt-soluble and salt- plus acid-soluble collagen were lower (P<.05) in steaks from the concentrate and CM groups than in steaks from the roughage group.

Diet had no effect (P>.05) on palatability traits and Warner-Bratzler shear values through SEP-3. At SEP-4 myofibrillar tenderness, connective tissue amount and overall tenderness scores were lower and Warner-Bratzler shear values were higher (P<.05) for steaks from the concentrate group. Scores for the CM group were intermediate. Steaks from the roughage group had the highest palatability and lowest shear values at SEP-4 (P<.05).

Longissimus muscle sarcomeres generally were longest for the concentrate and roughage groups and shortest for the control and CM groups. State of muscle contraction did not affect collagen solubility. Correlations were low between sarcomere length and total collagen (-.17), salt-soluble collagen (.29), acid-soluble collagen (-.03), overall tenderness (.39) and actual fat thickness (.17).

Due to small palatability differences between nutritional regimens, data were stratified into tender (n=16) and less tender (n=15) groups based on overall tenderness. These two groups were different (P<.05) for myofibrillar tenderness, connective tissue amount, overall tenderness and Warner-Bratzler shear values; however, measures of collagen solubility were not different (P>.05) between the two groups. The tender group tended to have less total collagen (P<.10) and more salt-soluble collagen (P<.14). Acid-soluble collagen was not different between the two tenderness groups.