COLLAGEN CHARACTERISTICS IN BEEF FROM STEERS FINISHED ON FOUR DIFFERENT NUTRITIONAL REGIMES AND FOR DIFFERING LENGTHS OF TIME

by

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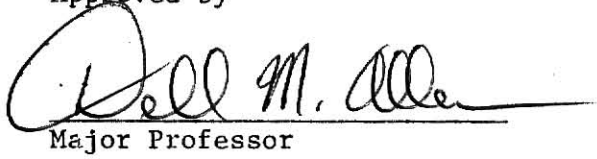

Major Professor

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INTRODUCTION

Meat quality, usually defined as factors that affect palatability, is a highly complex trait. Meat palatability depends primarily upon its tenderness, juiciness, and flavor. Of these, tenderness seems to be the most important and the principal test applied to cooked meat by the consumer.

Several researchers believe variations in meat tenderness are almost entirely due to changes in the state of the stromal and/or myofibrillar protein fractions (McClain et al., 1965a, 1970; Hill, 1966; Kruggel, Field, and Miller, 1970; Kruggel and Field, 1971; Goll et al., 1974; Dutson et al., 1976a; Marsh, 1977).

Collagen role in meat tenderness is not only important because of its relative abundance (estimated as much as 40% of the total body protein) but also it is the only protein in the body that has been shown to experience molecular changes during the maturation process in man and animals (Tanzer, 1973; Cross, Carpenter, and Smith, 1973; McClain, 1976a; Marsh, 1977). It is well documented that changes in collagen occur with increasing age (Verzar, 1963; Goll et al., 1964b,c,d; Harding, 1965; Bailey, 1969).

It is also widely accepted that these changes which occur with advanced age are associated with decreases in muscle tenderness (Hill, 1966; Zinn et al., 1970; Hunsley et al., 1971).

Limited work is found attempting to determine collagen differences between animals at similar chronological ages.

This study was designed with the following objectives:

(1) to study the influence of nutritional plane and/or length of time on feed on total collagen, heat labile collagen, and the proportions of acid and salt soluble intramuscular collagen of longissimus and biceps femoris muscle.

(2) to determine the relationship of the above traits to taste panel and shear measurement of tenderness.

REVIEW OF LITERATURE

This section is an effort to summarize basic features of the connective tissue system. Most of these generalizations are based on chapters from four books (Piez, 1967; Veiz, 1970; Bodwell and McClain, 1971; Forrest et al., 1975).

Connective Tissue

Muscle cells do not occur in the absence of connective tissue. Connective tissue is found throughout the body as a component of the skeleton, in the framework of organs, blood and lymph vessels, and in muscle tissue. It envelopes muscle fibers and bundles, and finally, the muscles themselves. The skin or hide is attached to the body by connective tissue. It also provides the body with a barrier against infective agents and is important in wound healing.

The connective tissues are generally characterized as having relatively few cells with large quantities of extracellular substances that vary from a soft gelatinous to a tough fibrous mass. Fibers are always found embedded within the extracellular substances thereby providing the structural connective tissue elements. Extracellular substance has differing properties in different tissue. In cartilage it has a rubbery consistency; in bone it is much tougher and is impregnated with calcium salts whereas it is a fluid without fibers in blood and lymph.

Connective Tissue Proper

Connective tissue proper consists of a structureless mass called ground substance in which cells and extracellular fibers are imbedded.

Connective tissue proper is characterized by the presence of distinct fibers and is usually referred to as fibrous connective tissue. Bone and cartilage are called supportive connective tissue because other tissues are attached to them, consequently providing the body with structural support.

Fibrous connective tissue contains two different types of cells: 1) fixed and 2) wandering cells. Fixed cells include fibroblasts, undifferentiated mesenchymal cells, and specialized adipose cells. Wandering cells are primarily found in body areas which have sustained injury. They include eosinophils, plasma cells, mast cells, lymph cells, and free macrophages.

Ground Substance

Ground substance is a viscous material containing soluble glycoproteins and substrates and end products of connective tissue metabolism.

The glycoproteins are commonly referred to as mucoproteins or mucopolysaccharides. Among the most plentiful mucopolysaccharides are hyaluronic acid and the chondroitin sulfates. Hyaluronic acid is a very viscous substance found in joints (sinovial fluids) and between connective tissue fibers. Chondroitin sulfates are found in cartilage, tendons, and adult bone. These two mucopolysaccharides and associated proteins act as lubricants, intercellular cementing substances, bone and cartilage structural matter, and as a barrier against infectious agents.

Extracellular Fibers

Tissue where the extracellular fibers are found in densely packed structures is referred to as dense connective tissue while those forming a loosely woven network are called loose connective tissue.

Dense connective tissue can also be classified by fiber arrangement. In dense irregular connective tissue the fibers are densely interwoven but in a random arrangement. However, in dense regular connective tissue the fibers are arranged in bundles lying parallel to each other.

The extracellular fibers include all three types of connective tissue fibers: collagenic, elastic, and reticular.

Collagen

Collagen is the most abundant single protein in the animal body (major fibrous element) making up 20 to 30% of total protein in most mammalian species. It is a glycoprotein that contains small quantities of sugars (galactose and glucose).

Collagen fibers are widely distributed in skin, bone, tendon, and the arterial walls. It also comprises the protein matrix for deposition of depot fat and supports and contains the individual muscle fibers (endomysium), muscle bundles (perimysium), and intact muscles (epimysium). Distribution and amounts of collagen are not uniform among skeletal muscles, with amount being generally a function of physical activity. Leg muscles contain more connective tissue than do supporting muscles (lumbar and thoracic regions).

McClain (1976a) reported that collagen acts as the major supporting framework of the body largely based on the high structural stability of the collagen molecules. This function is achieved by the unique molecular

configuration of collagen, the highly specific alignment of the molecules during extracellular aggregation, and more importantly by the formation of covalent crosslinks which give the fibers high tensile strength and resistance to chemical attack.

Elastin

Elastin is an extremely unreactive connective tissue which differs from collagen and reticulin in chemical composition, physical nature, and reactivity. It is a very minor component in mammalian skin, tendon, adipose tissue and muscle; but in certain tissues, particularly the ligaments of the vertebrae and the walls of the large arteries, elastin is present in large amounts.

Elastin is a rather rubbery protein; and aggregations of elastic fibers, such as in the ligamentum nuchae (cervical ligament), have a distinct yellow collor and the fibers are arranged in a parallel fashion. Elastic fibers are easily stretched, and they return to their original length when the tension is released. Unlike collagen, elastin does not undergo hydrolysis during cooking.

Reticulin

Reticulin is the least understood and studied of the fibrous connective tissue proteins. Reticulin is composed of small fibers that form delicate networks around cells, blood vessels, neural structures, and epithelium. Reticulin fibers are fine and wavy, have some degree of branching, and appear distinctly different than the more massive collagen fibers.

TABLE 1. PRINCIPAL AMINO ACIDS IN COLLAGEN AND ELASTIN

Amino acid	Collagen	Elastin
Glycine	33*	33
Hydroxyproline	up to 14	1 to 2
Hidroxylysine	1	none or --
Proline	12	10
Alanine	11	22
Tyrosine, histidine and sulfur a.a.	1	--
Tyrosine, tryptophan and sulfur a.a.	--	low or non existent
Desmosine and isodesmosine	--	present
Polar residues	18	5

* % of total amino acid residues

Notably absent from collagen is the essential amino acid, tryptophan (Table 1).

Hydroxyproline and hydroxylysine do not occur to any significant extent in other animal tissue, only in collagen. The hydroxyproline content of collagen is a relatively constant 13 to 14% and is absent in other meat proteins. Therefore, hydroxyproline chemical determinations are commonly used to quantitate collagen in animal tissues.

Of the three types of connective tissue fibers (collagen, elastin, and reticulin), collagen is most abundant and is abundantly found in muscle tissues. Therefore, it is felt that collagen is by far the most important connective tissue type affecting muscle tissue tenderness. Thus, further discussion in the remaining review of literature will be confined primarily to collagen and its role in meat tenderness.

Collagen Chain or Fibril

The native collagen fiber is synthesized through a highly ordered process of linear and lateral aggregation of thin, highly elongated macromolecules called tropocollagen, with a diameter of about 1.4 nm, a length of 280 nm, and a molecular weight of about 300,000 daltons. In the native state, tropocollagen macromolecules are composed of three hydrogen bonded chains intertwined in a characteristic helical configuration to produce stiff rods (Rubin et al., 1965).

The amino acids are linked together to form a single polypeptide chain in a left-handed coiled helix. Three of these polypeptide chains are intertwined in a right-handed coil in loosely coiled spring-like fashion to form a triple-stranded super helix. This triple-stranded

helix constitutes the tropocollagen molecule which is the structural unit of the collagen fibril.

Since glycine occurs as nearly every third amino acid along the polypeptide chain, it forms the first member of amino acid "triplets" along the collagen chain (-glycine-x-y-glycine-).

Proline is also present in large amounts in collagen with either hydroxyproline or proline occurring as the second amino acid in each "triplet" sequence along the polypeptide chain (x position). The third member (y position) may be any of the other amino acids present in collagen but frequently is alanine or hydroxyproline.

Numerous tropocollagen molecules are aligned in overlapping fashion to form the collagen fibril. Each tropocollagen molecule overlaps its lateral counterpart by one-fourth its length which accounts for the striated pattern or periodicity of the fibril. This quarter length overlap accounts for the predominant repeating distance of 64 to 70 nm observed in the native collagen fiber.

Tropocollagen units in the native fibrous form are not directly linked end to end but have "holes" of about 6 to 10% of a native fiber period interpose.

The collagen fibril varies from .3 to .4 μ in diameter, and a variable number of fibrils are combined in parallel array to form collagen fibers. Collagen fibers vary from 1 to 12 μ or more in diameter depending upon the number of fibrils present in parallel array. Individual collagen fibers are colorless, but fiber aggregation results in a white color.

Tropocollagen Components

Ultracentrifuge patterns of heat denatured tropocollagen show three separations identified as α, β, and γ component in order of increasing rate of sedimentation. The α component is identified as a single polypeptide chain with three in each tropocollagen molecule. Each has a molecular weight of about 100,000 (1/3 the molecular weight of undenatured tropocollagen). The β and γ components represent dimers and trimers, respectively, of α-chains and are formed by covalent cross-links between α components.

There are two forms of α components designated α_1 and α_2; β components (dimers), may be of three types: β_{11}, β_{12}, or β_{22} depending upon which chains, α_1 or α_2, are involved and upon the intra- or inter-molecular bonding character of the polymer. A variety of trimers, γ components, can be found: designated in a similar fashion by γ_{111}, γ_{112}, γ_{122}, and γ_{222}.

In most vertebrate tropocollagens, α_1 and α_2 occur in a stoichiometric ratio of 2:1. Thus, the noncross-linked tropocollagen molecule can be represented by the formula $(\alpha_1)_2\alpha_2$, where α_1 and α_2 are subunits of similar size but of different amino acid composition.

The β components, β_{11} and β_{12}, are formed by intramolecular (covalent bonds) crosslinks of α_1 and α_2. β_{22} is formed by intermolecular crosslinks of two α_2 components.

The β components are present in varying amounts, depending on the tissue and the method of extraction. The ratio of β_{12} to β_{11} components obtained from soluble collagen is usually about 4:1. Since the ratio would be exactly 2:1 if cross-linking were random, it can be concluded

that α_1 and α_2 behave differently, with α_2 components apparently having a greater potential for crosslinking. These differences in reactivity may be explained in part by following differences in amino acid composition:

1) α_2 is more basic (higher histidine content)
2) α_2 has large amounts of amino acids with hydrophobic side chains (valine, leucine, and isoleucine)
3) α_2 has lower content of the amino acids proline and hydroxyproline.

α Chains

Four distinct α_1 chains have been identified in various tissues (Martin et al., 1975; Bailey and Robins, 1976). Variation is due to slight differences in amino acid composition in the four types.

Table 2 shows the type, tissue location, and molecular composition of these four α_1 chains:

TABLE 2. TYPES OF α_1 COLLAGEN

Type	Tissue location	Molecular composition
I	Skin, tendon, bone, muscle	$(\alpha_1(I))_2\alpha_2$
II	Cartilage, intervertebral discs	$(\alpha_1(II))_3$
III	Fetal skin, cardiovascular system, synovial membrane, cardiac and skeletal muscle	$(\alpha_1(III))_3$
IV	Basement membrane	$(\alpha_1(IV))_3$

Bailey and Robins, 1976

Thermal Shrinkage of Collagen

This important collagen characteristic may be observed simply by exposing a bundle of collagen fibers to slow heating. At thermal shrinkage temperature (Ts) characteristic of the species from which the collagen was obtained, the fibers suddenly contract to 1/3 of their original length. This can occur at temperatures as low as 56 C in some fibrils and is usually complete in one half the muscle collagen fibrils at 61 to 62 C. Upon further heating for an extended time in the presence of moisture, collagen is hydrated and hydrolized, forming gelatin. Ritchey et al. (1963) pointed out that the Ts of collagen may explain "tightening of the bonds of connective tissue" surrounding muscle producing curling as steaks are broiled and the plump appearance of oven roasts. Gustavson (1957) has stated that the principal type of crosslinkage broken during thermal contraction of collagen is hydrogen bonding involving the hydroxyl group of hydroxyproline.

Collagen Crosslinks

Tropocollagen is secreted by fibroblasts into the extracellular space (connective tissue matrix). These large biosynthetic precursor molecules contain non-helical appendages at both the N- and C- terminal ends (this may facilitate the transmembrane movement of collagen molecules from the fibroblasts).

These submicroscopic fibrils grow in length and thickness through the accumulation of monomers by aggregation. Collagen fibers of newborn animals are usually small and embedded in viscous mucopolysaccharide

(ground substance). The fibers are held together by covalent crosslinks which increase in number as fibers mature.

Two types of crosslinks are known to occur: 1) intramolecular crosslinks within the tropocollagen molecule and 2) intermolecular crosslinks between molecules in the intact fibers.

Verzar (1963) postulated that "since collagen does not have a metabolic turnover," molecular movements eventually bring the collagen polypeptide chains nearer to each other, thus facilitating formation of crosslinkages. Harding (1965) further concluded that ester linkages occur in collagen and take part in intramolecular crosslinkages on the polypeptide chain. Traub and Piez (1971) reported evidence that epsilon-amino aldehydes were also involved.

McClain (1976a) reported the initial stage in the formation of intramolecular crosslinks involves oxidative deamination by a copper dependent enzyme, lysyl oxidase, of specific lysine or hydroxylysine residues located in the non-helical regions (N-terminal). The final product of this reaction is a δ-semi aldehyde of α-amino adipic acid called allysine. Within the collagen molecule, two of these aldehydes on adjacent chains condense to form an $\alpha;\beta$-unsaturated aldol condensation product. This aldol bond is very stable, being resistant to heat, salt, and acid extraction. But it only serves to crosslink the chains within molecules and thus cannot account for the increasing stability of the collagen matrix.

Intermolecular crosslinks are more important in stabilization of collagen fibers. Chemical studies by Bailey (1969) have demonstrated that these are aldimine type bonds. Tanzer (1973) reported that the

aldol condensation product with reconstituted fibrils slowly disappears during incubation, and a new substance is formed. This new substance, histidino-hydroxymerodes-mosine, is composed of equivalent amounts of the aldol condensation product, histidine, and hydroxylysine. Bailey (1969) designated this substance as "Fraction C". However, allysine can also condense with lysine or hydroxylysine to give the Schiff-base compounds called: hydroxylysinonorleucine and dihydroxylysinonorleucine (Robins, Shimokomaki, and Bailey, 1973). These two Schiff bases increase in quantity during the rapid animal growth period to a maximum, then slowly decrease to virtual absence at maturity (Bailey and Shimokomaki, 1971). This agrees with Bailey (1968) who has suggested that the Schiff-base crosslinks were only intermediates in the formation of the stable collagen fiber. Robins, Shimokomaki and Bailey (1973) proposed that as the animal matures, the labile Schiff-bases are replaced by more thermally stable crosslinks that are not reducible. They postulate that this would explain their decrease with increasing age and also the increased chemical and thermal stability of the bonds reflected in decreased collagen solubility and swelling.

Robins, Shimokomaki, and Bailey (1973) proposed that aldimide crosslinks derived from hydroxyallysine are stabilized by a mechanism involving the Amadori rearrangement. This was confirmed by Robins and Bailey (1975).

Collagen Turnover

Collagen is considered metabolically inert compared to other proteins (Verzar, 1963; Bailey and Robins, 1976) with early experiments having shown that collagen turnover is very slow (Gerber *et al.*, 1960; Popenoe

and Van Slyke, 1962). However, Dutson (1976b) has shown in mature ewes that intramuscular collagen turns over and collagen state can be altered even in mature animals.

Gerber, Gerber and Altman (1960) reported collagen turnover times as follows: tendon, < 110 days; muscle, 50 days; skin, 60 days; and kidney, 300 days.

Hydroxyproline Assay

Since collagen is the major component of connective tissue and essentially all hydroxyproline in animal tissues is in collagen, specific assays for this imino acid have been used to estimate the quantity of collagenous tissue in bovine muscle (Neuman and Logan, 1950; Prockop and Udenfriend, 1960; Woessner, 1961; Dahl and Persson, 1963; Bergman and Loxley, 1963; Serafini-Cessi and Cessi, 1964; Blumenkrantz and Asboe-Hansen, 1975).

Neuman and Logan (1950) developed a hydroxyproline determination that involves its oxidation to pyrrole - 2 - carboxylic acid, and then formation of a red chromophore on addition of Erlich's reagent (p-dimethylaminobenzaldehyde).

Numerous modifications of that method have been reported trying to improve repeatability of results and remove interference from other amino acids (Wierbicki and Deatherage, 1954; Prockop and Udenfriend, 1960; Woessner, 1961; Bergman and Loxley, 1963; Kivirikko et al., 1967; Blumenkrantz and Asboe-Hansen, 1975). Woessner's method (1961) gives recovery of one part of hydroxyproline in 4000 parts of other amino acids, but Bergman and Loxley (1963) report improved methodology

affording greater stability and chromagen sensitivity than obtained with Woessner's method.

Role of Connective Tissue in Muscle Tenderness

Collagen Contribution

Marsh and Leet (1966) proposed that meat toughness was due to the presence of connective tissue, other stromal proteins, and "actomyosin toughness" (attributed to the myofibrillar proteins). They called these two components "background toughness". They explained that in muscles with low connective tissue content, connective tissue is not related to tenderness. However, the contribution of connective tissue to toughness was greater in muscles of higher connective tissue content.

Dutson (1974) reported that total collagen in muscle tissue does not appear to be closely related with changes in tenderness that take place with animal age and post-mortem aging, but it does appear to be associated with differences in tenderness between muscles.

Carmichael and Lawrie (1967) reported that when the degree of post-mortem contraction is not a major variable, muscles differ in toughness according to their location; and given muscles vary in toughness according to animal age. Such differences might seem explicable on the basis of the relative connective tissue content and, in particular, by the concentration of its main constituent, collagen.

Krugger et al. (1970) reported beef muscle tenderness is very complex and cannot be simply resolved by studying a single component since many factors are involved and contribute to variations in beef tenderness. They divided beef tenderness affectants into three broad classifications: 1) ante-mortem (age and genetic, feeding and management

practices); 2) post-mortem (temperature and length of storage, time after slaughter, methods of trimming, cutting, and cooking methods); and 3) structural (molecular structure of the collagen).

Dutson et al. (1976a) pointed out that two of the major contributors to muscle tenderness are: 1) the "state" and content of connective tissue; and 2) the structure and stage of contraction of the myofibrils.

Collagen Changes

Studies on meat animals have shown that the total muscle collagen does not increase with increasing chronological age (Wilson et al., 1954; Goll et al., 1963). Herring (1967) reported that collagen content did not differ significantly in longissimus muscles of beef animals of A, B, and E maturity groups. However, the semi-membranosus had a higher collagen concentration ($P < 0.05$) in E than A and B maturity groups. He postulated that this reflects an increased rate of "semi-membranosus maturation".

The decrease in tenderness as animals age may result from connective tissue bonding changes, not from total connective tissue increase, due to exercise. Although total connective tissue apparently changes very little after maturity, the number of intermolecular crosslinks in the collagen fibrils increases. This results in decreased collagen solubility and increased resistance to shearing or chewing action.

A substantial muscle toughening in beef becomes evident at about 30 months of age. Beyond this time a further gradual toughening occurs, but the rate of change becomes progressively slower with advanced age (Forrest et al., 1975).

In general, the muscles of very young animals are much more tender than those of the very mature animal, but the changes are not linear with age. During the rapid phase of growth, tenderness seems to actually increase in some animals with time. It is likely that the rapid development of muscle fiber size "dilutes" the existing connective tissue. Thus, mature beef animals (12 to 18 months of age) often have more tender meat than the growing calf (6 to 9 months of age). Shimokomaki et al. (1972) suggested that the most tender beef should be obtained at about 12 to 14 months. Henricksen and Moore (1965) confirmed this. Gersh et al. (1949) (cited by Vognarová et al., 1968) explained greater amount of collagen and elastin in veal on the basis of the absolute quantity of connective tissue in the muscle throughout the life of the animal. With increasing age, only the thickness of connective tissue fibers increased. On the other hand, Hiner et al. (1953) reported that muscle fiber size increases directly with age. Thus, the proportion of connective tissue to total muscle protein decreases.

A number of investigators have shown that muscle fiber size increases with maturity. McMeekan (1940-41) reported by Wilson et al. (1954) has shown in hogs that from birth until 28 weeks of age the muscle fibers increase over eight times in diameter and ten times in cross-sectional area, thus increasing intracellular protein. At the same time, the absolute amount of connective tissue remains relatively constant throughout life, and small connective tissue fibers coalesce into stronger fibers with age (Porter, 1951). Goll et al. (1964b) and

Hill (1966) reported an increase in the number and strength of the crosslinkages of intramuscular collagen in meat animals as they age.

Although the above discussion of age-associated changes in tenderness has included reference to chronological age, it should be emphasized that the changes reflect the physiological environment within the animal (Forrest et al., 1975).

Correlations Between Collagen and Measures of Muscle Tenderness

Conflicting results have occurred in the literature in relating solubilized collagen to measures of meat tenderness.

Loyd and Hiner (1959) found a negative correlation between collagen content and sensory panel tenderness ratings (r = -.70 to -.90). Parrish et al. (1962) found a correlation coefficient between mean hydroxyproline and sensory tenderness for all cuts examined of -.84. Penfield and Meyer (1975) heated two kilogram round portions in a water bath at rates comparable to oven roasting at 93 C or 149 C to study connective tissue changes in semi-tendinosus cores. They reported increased ($R \leq 0.01$) hydroxyproline solubilization as end point temperature increased. As percentage of solubilized hydroxyproline increased, shear values decreased (r = 0.704**). Cross et al. (1973) found a significant relationship between percentage soluble collagen and sensory panel ratings for amount of connective tissue.

McClain et al. (1965b) found that the amount of collagen in raw or cooked beef was not related to shear values of the cooked meat. Bayne et al. (1971) reported that alkali insoluble collagen content of cooked meat was not related to shear values but that it was related to taste

panel tenderness score (r = .657*) and chew count (r = .643*). Paul, McCrae and Hoffered (1973) reported correlation coefficients for percentage solubilized hydroxyproline with shear force measurements of .09 (non significant) in the semitendinosus and -.45 (significant) in the biceps.

A low collagen-tenderness relationship has been shown by Hershberger et al. (1951), Wierbicki and Deatherage (1954), Ritchey et al. (1963), and Carpenter et al. (1963). On the other hand, some workers (Adams, Harrison, and Hall, 1960; Parrish et al., 1962) reported a significant negative association between collagen content and meat tenderness.

Herring et al. (1967) found that collagen solubility was significantly (R 0.01) correlated with taste panel tenderness for both longissimus (r = .77) and semimembranosus (r = .81) muscle from animals of A, B, and E maturity. Total collagen content was not related to panel tenderness in either muscle.

Cross et al. (1973) has shown that elastin concentration was not related consistently to variations in tenderness of bovine muscles.

Obviously, previous work is in conflict as to the relationship that exists between collagen solubility and measures of muscle tenderness. Swatland (1976) reported that the correlation between cooked meat tenderness and objective and subjective evaluation of samples is sometimes very poor, particularly when a realistic comparison is made between commercial types of meat. He pointed out the inclusion of animals with varying age differences, such as veal and old-cow beef, as a way only to improve statistical respectability.

Collagen Solubility

Several workers (Hill, 1966; Carmichael and Lawrie, 1967; Vognarová et al., 1968; Hunsley et al., 1971) found a decrease in collagen solubility in nondenaturing solvents when attempting to correlate properties of collagen with meat tenderness.

Collagen is more soluble in young animals and becomes less soluble as animal age increases. Thus, collagen solubility is an indicator of the number of crosslinks.

Schaub (1963) studied collagen aging in rat skeletal muscle connective tissue by determining the percentage of the total collagen solubilized in Ringer's solution for 10 min at 65 C. He reported 30 to 40% soluble hydroxyproline values in striated muscle from 10 month old rats, decreasing to 3 to 4% in older animals.

Goll et al. (1964b) reported as chronological age increased in bovines, collagenous residue susceptibility to collagenase digestion decreased and that less hydroxyproline was released by heating in phosphate buffer at 60, 65, 70, and 100 C (Goll et al., 1964c; 1964d). This study indicated occurance of more frequent or stronger crosslinks within and among tropocollagen molecules from older animals evidence by a decreasing rate of solubilization.

Vognarová et al. (1968) reported veal collagen was twice as soluble as beef collagen upon heating in one-fourth strength Ringer's solution.

Field, Pearson and Schweigert (1970) measured the yield of heat labile collagen from the toughest (shear force 6.41 ± 1.65) and most tender (shear force 3.17 ± .47) longissimus muscles of cutter and canner cows. Total collagen content of epimysial connective tissue was similar

in tough vs tender muscles. However, yield of labile collagen was higher ($P < .05$) in the tender muscles (6.25%) than in the less tender (5.35%). Results suggest a positive relationship between tenderness and yield of heat labile collagen.

Cross, Carpenter and Smith (1973) concluded that percent soluble collagen was significantly related to the connective tissue toughness contribution as assessed by a sensory panel.

Solubility of collagen reflects the various aspects of intra- and intermolecular crosslinking between the α-chains of the tropocollagen molecules. A proportion of collagen is extractable by a neutral salt solution, another portion requires dilute acid to make it soluble. "Mature" collagen or the residue which resists such solvents can be solubilized by enzymic, physical, or more severe chemical means. Piez et al. (1963) showed through labeling studies that the salt extractable fraction is a relatively small part of the total, and this is in transition to a less easily extractable, more highly intramolecular crosslinked material.

It is clearly demonstrated in the literature that total collagen in muscle is not consistently related to its tenderness. It is also clearly shown that the amount of soluble or labile collagen decreases as the animal matures, and it is well established that tenderness decreases with biological maturity.

Animal Nutritional Status and Collagen

Collagen is not only affected by physical forces such as temperature increases or irradiation but also by factors which produce or prevent

crosslink formation. Several nutritional factors are known to play an important role in collagen solubility. McClain (1976b) reported that levels of ascorbic acid, iron, vitamin D, copper, and zinc, in addition to dietary type of carbohydrate, and diet restriction can influence collagen crosslinking. McClain (1976a) reported that animals maintained on a low protein diet have more reducible collagen crosslinks than those maintained on protein surplus diets. When animals are starved as short as 24 hr, there were 50 to 60% fewer crosslinked components. This suggests that dietary factors can influence collagen with resulting influence on muscle quality.

Little applicable work has been done in bovines comparing collagen characteristics as related to meat tenderness when animals are fed on different levels of nutrition and different lengths of time prior to slaughter.

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MATERIAL AND METHODS

Thirty-two crossbred steers of known origin born at the U.S.D.A. Meat Animal Research Center at Clay Center, Nebraska were castrated at birth and fed on a bromegrass and bluestem pasture with their dams until weaning at six months of age. For the next 75 days the steers were fed a ration of approximately 65% corn silage (International reference no. 3-02-824), 15% alfalfa haylage (3-08-151) and 20% grain and soybean supplement (4-02-932 and 5-04-604). At the end of this period each steer was implanted with Ralgro (36 mg zeranol). All steers were wintered on corn silage and alfalfa haylage (ration 1, table 3) for 134 days and then pastured 133 days on bromegrass and native bluestem.

Eight randomly assigned grass-fed animals (Group I) were slaughtered directly off pasture at the ending of the grazing season. After a six-day adjustment period, the other animals were randomly assigned in groups of eight to the following slaughter groups. Group II was fed 49 days and Group III 98 days on a high concentrate ration (ration 3, table 3). Group IV was fed 98 days on a high silage ration (ration 2, table 3).

Animals in Group I were approximately 18 months old at slaughter. Groups III and IV were a maximum of 98 days older. The steers were weighed off test and trucked to the meats laboratory at Kansas State University and slaughtered 24 hours later. About 1 hr post-mortem the right side of each animal was chilled at 3 C until carcass fabrication at 48 hr post-mortem.

TABLE 3. RATION INGREDIENTS AND APPROXIMATE ANALYSIS

Ingredient	International reference no.	Ration 1	Ration 2	Ration 3
Corn silage, %	3-02-824	48.0	40.0	0.0
Alfalfa haylage, %	3-08-151	50.0	20.0	20.0
Cracked corn, %	4-02-932	0.0	36.0	75.2
Supplement[a], %		2.0	4.0	4.8
Approximate ration composition, dry matter basis[b]				
Dry matter, %		44.9	60.0	81.2
Crude protein, %		14.6	13.0	13.0
Metabolizable energy, mcal/kg		2.18	2.84	3.11

[a]Soybean meal (ref. no. 5-04-604) supplement plus calcium, phosphorus, vitamin A and chlortetracycline. Block salt and a mixture of 1/3 loose salt, 1/3 limestone and 1/3 dicalcium phosphate were also available (free access).

[b]Nutrient composition based on tabular values (N.R.C., 1963) supplemented with limited approximate analysis.

Sample Location

The biceps femoris (BF) and longissimus dorsi (LD), from the 13th thoracic through the 5th lumbar vertebral locations, were excised intact from the carcass. Each muscle was cut into a posterior and anterior half, and the latter was fabricated into 3.0 cm thick steaks. Beginning at the mid-point end and continuing anteriorly, steaks were removed as follows: 1st steak, taste panel and shear force determination; 3rd steak, collagen studies.

All steaks were vacuum packaged, frozen and stored at -26 C until evaluated.

Shear Force and Taste Panel

Steaks were thawed at 2 C for 24 hr, unpackaged, rinsed, blotted, and broiled in a rotary gas oven at 163 C to an internal temperature of 66 C as determined by a cooking thermometer.

Taste panel tenderness of the LD muscle was evaluated by a six-member trained panel using a nine-point hedonic scale (Kramer and Twigg, 1966). Panelist selection and training was done by serving samples of differing tenderness and evaluating individual sensitivity by triangle comparisons (Amerine et al., 1965; Kramer and Twigg, 1970).

Panelists were randomly distributed in individual booths and served half of a warm 1.91 cm diameter core (cut perpendicular to fiber axis). Panelists were instructed to chew each sample as necessary but not to swallow it and to rinse their mouth with water between samples. Five samples per session were randomly served with no more than two sessions per day. A warm-up sample of U.S.D.A. Choice LD was provided

each panelist prior to each sampling session, and a second U.S.D.A. Choice LD sample was included with test samples as a "hidden" reference.

Using the same LD steak, six 1.25 cm diameter cores were removed with a drill press unit (Kastner and Henrickson, 1969) and shear force assessed using a Warner-Bratzler shear. This was also done for the BF muscle.

Sample Preparation for Collagen Determinations

Frozen tissue was ground with liquid nitrogen in a Waring Blender, soaked with cold distilled water in an erlenmeyer flask for three days (five to six water changes) and freeze dried. All freeze dried samples were kept in a screw cap tube at 0 C.

Heat Labile Collagen

Determination of the heat labile collagen was done according to a modification of the procedure of Hill (1966). A total of 20 ml of one-fourth strength Ringer's solution was placed in a 50 ml centrifuge tube in addition to 100 to 150 mg of freeze dried sample. The samples were heated in a water bath for 1 hr at 66 C and then cooled in an ice bath. Tubes were centrifuged at 3000 x g for 15 min. One ml of supernatant was placed in a 25 ml screw-top culture tube (teflon lined caps) and brought to 6 N acidity with concentrated HCl. Tubes were sealed under a stream of nitrogen gas and hydrolyzed for 12 hr at 121 C. Samples were dried under a stream of air in a 60 C water bath. Residue was analyzed for hydroxyproline.

Hydroxyproline Determination

Hydroxyproline was determined by a modification of the procedure of Bergman and Loxley (1963) (Appendix A). One-half ml distilled water was added to the air dried samples and well mixed. Following mixing 0.81 ml isopropanol was added and mixed, then 0.4 ml of oxidant solution (Chloramine T + buffer) was added, mixed, and treated samples were allowed to stand for five minutes at room temperature. Five ml of Ehrlich's reagent per tube was added, well mixed, and the tubes heated for 25 minutes at 60 C in a water bath. Tubes were cooled in running tap water, and then the absorbance was measured in a Gilford spectrophotometer at 560 nm using a 1 cm flow-through cuvette using isopropanol as a blank. Hydroxyproline was calculated directly from a standard curve prepared prior to each run.

Salt and Acid Soluble Collagen

Salt soluble collagen. Freeze dried muscle samples (500 mg) were placed in a 50 ml centrifuge tube and 10 ml of cold 1.0 M NaCl with .05 M Tris (pH 7.5) added. Tubes were shaken for 48 hr at 4 C and centrifuged at 40,000 g for 30 min. One ml aliquots were placed into 10 ml ampules and one ml of concentrated HCl added. Ampules were sealed under vacuum and hydrolyzed prior to hydroxyproline analysis.

Acid soluble collagen. Residue from the salt soluble fraction was washed (shaker) with distilled water for one hour at 4 C, centrifuged at 40,000 g for 30 min and supernatant discarded. Pellets were extracted with 10 ml of cold .5 M Acetic acid (shaker) for 48 hr at 4 C. Tubes were centrifuged and aliquots taken as before.

Total Collagen

Samples of 35 to 40 mg of freeze dried muscle were placed in 50 ml screw-top culture tubes with teflon lined caps and 30 ml of 6 N HCl added. Tubes were sealed under a stream of nitrogen gas and hydrolyzed for 12 hr at 121 C. Volume was made up to 50 ml, and two ml aliquots were dried as before under an air stream.

Statistical Analysis

One-way analysis of variance was done on all carcass traits and collagen determinations, and least significant difference test was applied to means according to Snedecor and Cochran, 1967. Overall simple correlation coefficients were determined between all traits.

RESULTS AND DISCUSSION

Effect of Nutritional Plane on Salt Soluble Collagen

Treatment had an effect ($P<.01$) on yield of salt soluble collagen from LD muscle (table 4). LD muscle from steers finished on grass had a lower ratio of salt soluble to total collagen than did LD from the other nutritional treatments (table 5).

Collagen extractable by neutral salt solutions represents "the most newly synthesized collagen" and yields more α -chains upon degradation than other collagen components (Weiss, 1976). Kruggel and Field (1971) indicated that decrease in quantity of α -chains is accompanied by an increase in muscle toughness. McClain (1976) related that quantity of salt soluble collagen from rat skin was reduced by more than 35% for low protein (8%) diets in relation to controls (25%), suggesting decreased collagen biosynthesis in animals on low protein diets. When cattle finished on grass are slaughtered directly off pasture at the end of the grazing season, some restriction in their protein intake is possible, thus contributing to lower synthesis of new collagen. Unlike the LD muscle, nutritional treatment had no effect on yield of salt soluble collagen from the BF (table 4). The BF muscle is an early maturing muscle (Berg and Butterfield, 1976) while the LD is an average maturing muscle, thus, biosynthesis of new collagen in the BF may be more consistent across nutritional treatments.

Effect of Nutritional Plane on Acid Soluble Collagen

Acid soluble collagen from the LD was not affected by the nutritional regimes of this study, but a significant difference was found in BF

TABLE 4. ANALYSIS OF VARIANCE FOR COLLAGEN TRAITS, TENDERNESS SCORE, SHEAR FORCE, MARBLING AND FINAL GRADE FROM *LONGISSIMUS* AND *BICEPS FEMORIS* MUSCLE

	Treatment	Error
Degrees of freedom	3	28
	Mean Square	
Longissimus dorsi		
Salt soluble collagen	.250***	.046
Acid soluble collagen	.076	.073
Salt + acid soluble collagen	.424*	.148
Heat labile collagen	30.14	32.08
Total hydroxyproline	1.16**	.149
Taste panel tenderness	3.40**	.989
Shear force	.558	.451
Biceps femoris		
Salt soluble collagen	.024	.022
Acid soluble collagen	.033**	.008
Salt + acid soluble collagen	.088	.044
Heat labile collagen	4.16	2.26
Total hydroxyproline	1.51	1.12
Shear force	1.31	1.50
Carcass traits		
Marbling	45638.0***	8937.6
Final grade	8.78**	2.34

*$P \leq .054$
**$P < .05$
***$P < .01$

TABLE 5. EFFECT OF FEEDING REGIME ON BEEF LONGISSIMUS AND BICEPS FEMORIS COLLAGEN COMPONENTS (MEANS), TENDERNESS SCORES, SHEAR FORCE, MARBLING AND GRADE

	FEEDING REGIME			
	Grass	49 day High conc.[a]	98 day High conc.[a]	98 day High forage[b]
Longissimus dorsi				
Salt soluble[c]	.44[d]	.71[e]	.75[e]	.86[e]
Acid soluble[c]	.87	.89	1.08	.90
Salt + acid soluble[c]	1.31[d]	1.61[de]	1.83[e]	1.76[e]
Heat labile[c]	24.34	20.72	23.38	20.43
Total hydroxyproline[g]	.667[d]	.538[e]	.415[e]	.415[e]
Taste panel tenderness[h]	4.99[d]	5.60[de]	6.49[e]	6.11[e]
Shear force[i]	2.76	3.07	3.40	3.02
Biceps femoris				
Salt soluble[c]	.65	.62	.72	.73
Acid soluble[c]	.39[d]	.38[d]	.52[e]	.40[d]
Salt + acid soluble[c]	1.05	1.00	1.24	1.13
Heat labile[c]	9.88	9.82	8.64	8.56
Total hydroxyproline[g]	.631	.635	.543	.586
Shear force[i]	6.11	6.46	5.78	6.71
Carcass traits				
Loin eye area[j]	66.39[d]	76.19[e]	75.55[e]	74.06[e]
Marbling[k]	205[d]	262[de]	334[ef]	375[f]
Final grade[l]	4.1[d]	5.4[de]	6.1[e]	6.5[e]

[a] 80% concentrate - 20% forage ration

[b] 40% concentrate - 60% forage ration

[c] percentage of total collagen

[d,e,f] means within same row bearing same or no superscript letter do not differ ($P < .05$)

[g] percentage of freeze dried sample

[h] assessed by a trained taste panel (9 = extremely tender, 5 = acceptable, 1 = extremely tough)

[i] Warner Bratzler in kg

[j] in cm^2

[k] marbling scores (traces = 100 to 199; slight = 200 to 299; small = 300 to 399)

[l] final grade (standard = 1,2,3; good = 4,5,6; choice = 7,8,9)

muscle (table 4). BF from animals fed 98 days on a high concentrate ration had a higher ratio of acid soluble to total collagen than other treatments (table 5). Kruggel et al. (1970) found epimysial acid soluble collagen contained more crosslinks when obtained from tough muscles than when obtained from tender muscles. Weiss (1976) reported that biologically older collagen has a greater number of intramolecular crosslinks as well as some intermolecular crosslinks, thus, forming dimers and trimers not soluble in neutral salt solution but soluble in dilute organic acids (i.e., acetic acid). Decreased acid solubility of BF collagen may be due to greater physiological maturity of the muscle (Zinn et al., 1970).

Mean LD salt soluble collagen across treatments (.69%) was similar to that from BF (.68%). However, a higher mean value of acid soluble collagen across treatments was realized from the LD (.93%) than from the BF (.42%). This suggests greater intermolecular crosslinking present in the BF that is more resistant to breaking than in the LD. This would agree with the suggestion of Zinn et al. (1970) that muscles from the hind limb are in a more advanced state of maturity than is the LD muscle. McClain et al. (1971) using semimembranosus muscle from animals at 14 to 15 months reported yields of .30 $\pm$ .12% for salt soluble and .48 $\pm$ .28% for acid soluble collagen. LD and BF yields of these collagen fractions reported here are similar.

Effect of Nutritional Plane on Total Combined Salt Plus Acid Soluble Collagen

Salt plus acid collagen from LD increased with increasing plane of nutrition and longer feeding (table 5). A similar trend was noted in

the BF muscle; however, differences were non-significant. Differences in combined salt and acid soluble collagen in the LD were due mostly to differences in the yields of salt soluble collagen from that muscle.

Effect of Nutritional Plane on Heat Labile Collagen

No differences were found in the percentages of heat labile collagen between nutritional regimes for either LD or BF muscles (table 5). Yields of heat labile collagen from BF muscle agree with those reported by Hill (1966); Paul, McCrae and Hoffered (1973); and Cross et al. (1973).

Effect of Nutritional Plane on Total Collagen

LD muscle from grass finished beef had significantly more hydroxyproline than beef finished on the other nutritional treatments (table 5). No significant differences between treatment were found in the BF muscle (table 4), but in both muscles total hydroxyproline tended to decrease as plane of nutrition increased. As animals were placed on a higher nutrition plane after being removed from grass, rapid growth occurred most of which was muscle enlargement (Allen et al., 1977). LD muscle area (L.E.A.) increased significantly with increased plane of nutrition and time on feed (table 5). This suggests that at this rapid growth phase, myofibrillar and sarcoplasmic proteins were being synthesized in greater amounts than was collagen. Thus, a significantly lower proportion of collagen was present in muscle from animals after 49 or 98 days on a high plane of nutrition. These findings agree with Reddy (1971) who reported that beef animals fed on low energy diets had 23% more LD hydroxyproline than did animals on high energy diets. He reported mean LD fiber diameter to be greater (indicating more hypertrophy) in

animals on the high energy diet. Marbling (intramuscular fat) level increased significantly with increasing time on feed and plane of nutrition in our study (table 5). Carmichael and Lawrie (1967) reported that increasing amounts of myofibrillar and sarcoplasmic proteins and increasing fat levels act as a dilution factor on total collagen apparently lowering the concentration of hydroxyproline in the tissue.

Hydroxyproline makes up a consistent 13 to 14% of total collagen (Newman and Logan, 1950; Hunstley et al., 1971). This fact allows total collagen to be calculated by determining the amount of hydroxyproline and multiplying by a constant (7.25 according to Hunstley et al., 1971; 7.46 according to Neuman and Logan, 1950).

Effect of Nutritional Plane on Taste Panel Tenderness and Shear Force

LD steaks from animals finished on grass were rated by a taste panel as being less tender ($P<.05$) than steaks from animals fed grain or silage for 98 days. Tenderness as measured by the taste panel increased with increasing plane of nutrition and time on feed (table 5). These results agree with the findings of Kropf et al. (1975) that tenderness, flavor, and overall acceptability was higher for long-fed animals and lowest for grass-fed animals. Plane of nutrition and length of feeding period have definitely improved taste panel palatability ratings of beef LD. When animals are taken from a relatively low nutrition plane and placed on a high plane of nutrition and fed for a period of time, rapid growth generally occurs. During this rapid growth and fattening phase, LD taste panel tenderness scores are improved.

Correlations

No significant relationship was observed between taste panel tenderness or shear force and the various collagen characteristics studied for the biceps femoris muscle. Only longissimus salt soluble collagen (table 6) was correlated with taste panel tenderness ($P<.05$).

TABLE 6. SIMPLE CORRELATION COEFFICIENTS FOR LONGISSIMUS MUSCLE

	Taste panel tenderness score	Warner-Bratzler shear force	Marbling	Final grade
Salt soluble	.43*	-.13	.45**	.42*
Acid soluble	.12	.12	.03	-.08
Salt + acid	.33	-.01	.30	.21
Heat labile	-.21	.17	.05	.06
Total collagen	-.31	-.09	-.44*	-.42*

**P <.01 (r 0.01 = .449)
*P <.05 (r 0.05 = .349)

Cover et al. (1962), Bouton and Harris (1972), and Cross et al. (1973) reported that shear force values are more closely related to muscle fiber properties than to connective tissue components in cooked meat. This would appear to be true in the results of this study also. Herring et al. (1967) reported that simple overall correlation coefficients were highly significant for collagen solubility and panel tenderness (r = .77) for longissimus muscle from A, B, and E maturity group. However, within maturity group correlations were low and nonsignificant as well as the relationship between total collagen content with panel tenderness. Results of this study conflict with these workers findings since a significant relationship was found between salt soluble collagen

and taste panel tenderness in beef longissimus muscle from steers all within the A maturity group. Parrish, Bailey, and Naumann (1962) found no significant correlation between tenderness and hydroxyproline from longissimus muscle of choice and standard beef. McClain et al. (1965) found no differences in alkali-insoluble collagen between tough and tender longissimus muscle.

As time on feed and plane of nutrition increased, an increase in marbling and final grade was observed (table 5). Since salt soluble collagen also increased in the same way, a correlation between salt soluble collagen and these traits would be expected. Longissimus salt soluble collagen was related to marbling (r = .45**) and final grade (r = .42*). As presumed, total hydroxyproline was also significantly related with marbling (r = -.44*) and final grade (r = -.42*) for longissimus muscle.

Summary

The objective of this study was to determine the influence of nutritional plane and/or length of time on feed on some characteristics of collagen and to determine their relationship to tenderness as measured by taste panel and shear force measurement.

Thirty-two crossbred steers of known origin were randomly assigned to four different nutritional regimes. Eight animals were slaughtered directly off pasture (grass-fed). Eight animals were fed 49 days and eight 98 days on an 80% concentrate - 20% forage ration. The last eight were fed 98 days on a 40% concentrate - 60% forage ration. Animals were approximately 18 months old when slaughtered. After 48 hr chill at 3 C,

longissimus and biceps femoris muscles were sampled from the right side of each animal.

LD salt soluble collagen was lower ($P < .05$) in grass-fed steers, but no nutritional regime difference was found for BF. BF from steers fed 98 days on a high concentrate ration had a higher percentage ($R < .05$) acid soluble of total collagen, but LD showed no difference. Salt plus acid soluble collagen from LD increased ($P < .05$) with increasing plane of nutrition and longer feeding. BF revealed a similar but non-significant trend. Nutritional treatment had no effect on heat labile collagen for either LD or BF. Increasing plane of nutrition and longer time on feed decreased ($P < .05$) total collagen hydroxyproline from LD and a like but non-significant trend was observed in BF. Higher plane of nutrition and longer feeding improved ($R < .05$) taste panel tenderness. Shear force was not affected by nutritional regime. A significant correlation was found ($r = .43*$) between salt soluble collagen and taste panel tenderness score for the LD. Salt soluble collagen and total hydroxyproline were correlated with marbling ($r = .45**$, $r = .42*$) and final grade ($r = -.44*$, $r = -.42*$).

LD steaks from steers finished on a high plane of nutrition for up to 98 days have higher yields of salt soluble collagen, lower proportion of total collagen hydroxyproline and preferred taste panel ratings. This study suggests that higher nutritional plane improves tenderness perhaps partly by decreasing total collagen percentage in the LD muscle but also by a greater percentage being present in the salt soluble state.

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APPENDIX A

Procedure for Spectrophotometric Determination of Hydroxyproline (Bergman, I. and Loxley, R., 1963. Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. Anal. Biochem. 35:1961).

Preparation of Standard Solution

Dissolve 50 mg L-hydroxyproline in 500 ml of 0.001 N HCL. This solution (100μ g/ml) can be stored in a glass stoppered bottle in a refrigerator (4 C).

Preparation of Reagent Solutions

Buffer solution (pH 6.0)

57.0 g sodium acetate trihydrate ($NaC_2H_3O_2 \cdot 3H_2O$)

37.5 g trisodium citrate (2 H_2O) ($Na_3C_6H_5O_7 \cdot 2H_2O$)

5.5 g citric acid (H_2O) ($C_6H_8O_7 \cdot H_2O$)

385 ml of isopropanol

Dilute above to 1000 ml with distilled water. This solution is stable indefinitely.

Oxidant Solution

1. Prepare a 7% weight/volume aqueous solution of chloramine T (sodium salt of p-toluene sulfon-chloramide) (Prepare fresh)

2. Just before each series of determination, mix one volume of Chloramine T with four of buffer solution (about 0.5 ml per analytical value is required)

Erligh's Reagent Solution

1. Dissolve p-Dimethylaminobenzaldehyde (p-dmab)[1] in 60% perchloric acid in the proportion of 2 g of p-dmab to 3 ml of acid (Prepare daily)

2. Just before the start of a series of determination, mix the above solution with isopropanol in the proportion of 3 volumes of p-dmab solution to 13 volumes of isopropanol (5 ml needed per sample plus an excess for dispensing system)

Procedure for hydroxyproline assay

1. Add 0.5 ml of distilled water to test tube for air dried samples[2] or 0.5 ml of the hydroxyproline standard solutions.

2. To each tube add 0.8 ml of isopropanol and mix well.

3. Add 0.4 ml of oxidant solution mix, and allow to stand 5 minutes.

4. To each tube add 5 ml of the Erlich's reagent solution, mix very well and cap the tubes.

5. Heat the tubes for 25 min at 60 C in a water bath. Cool tubes for 3 to 4 min in running tap water.

6. Measure absorbance against isopropanol at 560 nm.

[1] best results were obtained with p-dmab from Matheson Coleman and Bell

[2] % standard recovery for air dried procedure ranged from 95.5 to 98.6%

Note: % NaCl added up to 10% in the hydroxyproline analysis had no effect on absorbance readings

APPENDIX TABLE A. EFFECT OF FEEDING REGIME ON PERCENTAGE OF SALT AND ACID SOLUBLE COLLAGEN FOR LONGISSIMUS MUSCLE

FEEDING REGIME			
Grass	49 Day high conc.[a]	98 Day high conc.	98 Day high forage
	Salt soluble[b]		
.445	.588	.821	.645
.510	.423	.761	.665
.467	.804	1.149	1.060
.384	.740	.511	1.419
.410	.441	.685	.740
.518	1.177	.627	.722
.262	.764	.609	1.016
.545	.774	.812	.610
.443[c]	.713	.747	.860
.091	.240	.194	.282
	Acid soluble[b]		
.839	1.052	1.260	1.353
1.021	.851	1.871	.494
.720	.845	1.148	1.186
.626	1.233	.825	.927
1.174	.640	.985	.864
1.072	.785	.802	.638
.672	.868	.640	.926
.808	.878	1.109	.812
.866	.894	1.080	.900
.201	.178	.379	.275

[a] high concentrate ration

[b] expressed as a percentage of the total collagen

[c] mean and standard deviation

APPENDIX TABLE B. EFFECT OF FEEDING REGIME ON PERCENTAGE OF SALT AND ACID SOLUBLE COLLAGEN FOR BICEPS FEMORIS MUSCLE

FEEDING REGIME			
Grass	49 Day high conc.[a]	98 Day high conc.	98 Day high forage
	Salt soluble[b]		
.619	.660	.746	.886
.596	.464	.646	.863
.514	.689	.649	.603
.492	.605	.559	.471
.717	.517	.929	.860
.788	.625	.758	.502
.836	.729	.550	1.028
.604	.646	.919	.623
.646	.617	.720	.729
.124	.088	.147	.205
	Acid soluble[b]		
.271	.290	.468	.456
.320	.280	.544	.348
.278	.454	.454	.377
.595	.357	.397	.389
.380	.390	.659	.458
.458	.488	.403	.395
.430	.480	.703	.444
.410	.340	.525	.369
.393	.385	.522	.404
.107	.082	.119	.042

[a]high concentrate ration

[b]expressed as a percentage of the total collagen

[c]mean and standard deviation

APPENDIX TABLE C. EFFECT OF FEEDING REGIME ON PERCENTAGE OF HEAT LABILE COLLAGEN FOR LONGISSIMUS AND BICEPS FEMORIS MUSCLES

	FEEDING REGIME		
Grass	49 day high conc.[a]	98 day high conc.	98 day high forage
	Longissimus		
20.52[b]	19.33	25.76	18.99
27.92	14.88	18.82	10.18
21.15	32.19	18.02	24.41
13.38	19.54	21.66	16.58
22.28	20.55	33.80	25.98
31.28	20.53	18.87	19.29
33.17	19.89	20.15	24.23
25.06	18.84	29.95	23.79
24.34[c]	20.72	23.38	20.43
6.42	4.97	5.86	5.29
	Biceps		
6.84	9.13	8.54	8.77
8.80	7.36	11.48	8.37
11.36	13.24	7.44	7.46
10.26	9.12	8.23	7.66
8.99	11.33	8.68	7.74
10.78	9.48	7.36	10.07
9.17	9.60	9.34	9.25
12.87	9.28	8.08	9.17
9.88	9.82	8.64	8.56
1.84	1.75	1.32	.92

[a] high concentrate ration

[b] expressed as a percentage of the total collagen

[c] mean and standard deviation

APPENDIX TABLE D. EFFECT OF FEEDING REGIME ON TOTAL CONTENT OF HYDROXYPROLINE FOR LONGISSIMUS AND BICEPS FEMORIS MUSCLES

Grass	FEEDING REGIME: 49 Day high conc.[a]	98 Day high conc.	98 Day high forage
	Longissimus		
.842[a]	.597	.439	.472
.615	.759	.449	.649
.791	.490	.301	.402
.665	.431	.638	.319
.553	.713	.336	.281
.535	.318	.414	.309
.666	.559	.388	.376
.672	.433	.356	.513
.667[c]	.538	.415	.415
.106	.149	.103	.124
	Biceps		
.676	.606	.595	.491
.621	.782	.535	.472
.824	.611	.569	.613
.546	.689	.730	.846
.572	.648	.365	.491
.509	.593	.629	.677
.619	.522	.461	.522
.684	.626	.457	.572
.631	.635	.543	.586
.098	.076	.114	.127

[a] high concentrate ration

[b] expressed as a percentage of the freeze-dried sample

[c] mean and standard deviation

APPENDIX TABLE E. EFFECT OF FEEDING REGIME ON TASTE PANEL RESPONSES AND SHEAR FORCE VALUES FOR LONGISSIMUS MUSCLE

FEEDING REGIME			
Grass	49 Day high conc.[a]	98 Day high conc.	98 Day high forage
	Taste Panel[b]		
5.50	6.17	7.08	6.25
4.83	4.92	6.00	7.08
6.08	5.42	7.17	6.00
6.00	4.33	5.92	5.50
4.83	5.83	5.75	4.33
6.41	7.08	6.25	5.33
2.91	5.25	6.17	7.67
3.33	5.83	7.58	6.75
4.99[c]	5.60	6.49	6.11
1.29	.83	.68	1.07
	Shear Force[d]		
2.35	3.20	3.58	2.71
2.43	3.73	3.29	2.37
2.45	2.66	2.28	3.42
2.06	3.82	4.64	3.10
3.02	2.91	3.14	4.40
2.27	2.13	4.26	2.83
4.23	2.95	2.84	2.69
3.29	3.16	3.21	2.62
2.76	3.07	3.41	3.02
.72	.55	.76	.64

[a] high concentrate ration

[b] evaluated on 9-point scale (9=extremely tender, 5=acceptable, 1=extremely tough)

[c] mean and standard deviation

[d] expressed in kg

APPENDIX TABLE F. EFFECT OF FEEDING REGIME ON SHEAR FORCE VALUES FOR BICEPS FEMORIS MUSCLE

Grass	49 Day high conc.[a]	98 Day high conc.	98 Day high forage
	FEEDING REGIME		
	Shear Force[b]		
5.59	5.36	5.50	7.85
5.55	9.70	6.61	6.93
5.33	6.85	4.85	5.50
4.58	3.59	6.34	6.64
6.43	7.49	6.98	6.89
6.18	5.89	6.24	7.57
6.74	6.36	4.37	5.51
8.49	6.40	5.36	6.79
6.11[c]	6.46	5.78	6.71
1.18	1.75	.91	.85

[a] high concentrate ration

[b] expressed in kg

[c] mean and standard deviation

COLLAGEN CHARACTERISTICS IN BEEF FROM STEERS FINISHED ON FOUR DIFFERENT NUTRITIONAL REGIMES AND FOR DIFFERING LENGTHS OF TIME

by

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Thirty-two crossbred steers of known origin, castrated at birth, were randomly assigned to four different nutritional regimes. All animals were fed on bromegrass and bluestem pasture and wintered on a growing ration. Eight animals were slaughtered directly off pasture at the end of the grazing season (grass-fed). Eight animals were fed 49 days and eight 98 days on an 80% concentrate - 20% corn silage ration. The last eight were fed 98 days on a 40% concentrate - 60% corn silage ration. Animals were approximately 18 months old when slaughtered.

The objective of this study was to determine the influence of nutritional plane and/or length of time on feed on some characteristics of collagen and to determine their relationship to tenderness as measured by taste panel and shear force measurement.

About one hour post-mortem the right side of each animal was chilled at 3 C until carcass fabrication at 48 hr post-mortem. The longissimus and biceps femoris muscles were excised and bisected. The anterior half was cut into steaks, vacuum packaged and frozen. One steak (first) was assigned for taste panel tenderness and shear force determination, and another steak (third) was assigned for collagen studies.

Nutritional regime improved ($P<.05$) taste panel tenderness. Steaks from steers on a high plane of nutrition and longer time on feed were most desirable. Shear force was not affected by nutritional regime. Salt soluble collagen from longissimus muscle was lower ($P<.01$) in grass-fed beef, but no difference was found in biceps femoris. Acid soluble collagen from biceps femoris was higher ($P<.05$) in 98 day high

concentrate ration animals. Salt plus acid collagen from longissimus increased ($P<.05$) with increasing plane of nutrition and length of feeding and a similar trend was noted in the biceps femoris. Nutritional treatment had no effect on heat labile collagen. Total collagen (hydroxyproline) from longissimus muscle decreased ($P<.001$) with increased plane of nutrition and length of feeding. A significant correlation ($r = .43^*$) was noted between salt soluble collagen in the longissimus muscle and taste panel tenderness.