EFFECTS OF DIETARY ETHANOL ON PERFORMANCE, CARCASS CHARACTERISTICS, AND MEAT TENDERNESS

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DEDICATION

To my wonderful family, your encouragement has given me persistence and determination to pursue a lifelong goal.

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CHAPTER I

INTRODUCTION

Skeletal muscle is a highly organized tissue that serves to provide locomotion to higher animals. Muscle, the major component of meat, also serves as an excellent source of food for man. The biochemical events between muscle and meat are complex and involve metabolic, physical and structural changes. Collectively, these intracellular changes parallel the development of rigor mortis and may elicit much variation in eating quality. In terms of consumer meal time values, it is conceivable that tenderness varies more widely than the characteristics of flavor and juiciness. To enhance consumer perception of beef relative to other muscle foods, appreciable interest exists in manipulating the contractile events of pre-rigor muscle and accelerating the aging process of beef musculature.

As first described by Locker and Hagyard (1963), pre-rigor beef muscles that are removed from their skeletal attachments shorten by up to sixty percent (60%) of the initial length if they are cooled at 2°C before entering the rapid phase of rigor onset. The length change associated with cold shortening is accompanied by a several fold toughening (Marsh and

Leet, 1966). Interference with the contractual design of muscle in a manner that would limit pre-rigor shortening would theoretically provide a mechanism to enhance beef quality.

Accelerating the aging process has also received considerable interest in recent years. Much of this interest has centered around developing processes which invoke early activation of the aging process by increasing the rate of degradation of the structural integrity of muscle. A serious limitation is the effective regulation of endogenous muscle components. According to Perkoff (1971) and Felix (1982) the sarcolemma can be made permeable to low molecular weight compounds. Additional evidence suggests that related compounds may induce a myopathic condition in the muscle cell (Song and Rubin, 1972). The administration of such compounds may be accomplished by infusing pre-rigor musculature at the time of slaughter or by supplementing a high energy ration during the feedlot phase (Kauffman, 1987). Although it has been proposed that the permeability of the sarcolemma may be enhanced to permit delivery of low molecular weight compounds to individual structural components, a compound that elicits a uniform, rapid and selective alteration has not been identified.

Evidence within the medical community suggest that ethanol has a direct effect on muscle (Urbano-Marquez et al, 1989). The acute and long term consumption of ethanol affects the permeability of the sarcolemma, the integrity of the contractile component and the regulation of contractile activity (Rubin et al., 1976; Puszkin and Rubin, 1976; and Urbano-Marquez

et al., 1989). Additionally, histological and enzymatic studies suggest that myopathy results from acute and chronic ethanol ingestions (Song and Rubin, 1972; Kahn and Meyer, 1969; Urbano-Marquez et al., 1985). Therefore, to consider the possibilities of maintaining and perhaps improving beef quality via ethanol supplementation, two phases will be investigated: 1.) Determination of the effects of increasing dietary ethanol supplementation on animal performance, carcass characteristics and meat tenderness; 2.) Determination of the effects of dietary ethanol on animal performance, carcass characteristics and meat tenderness over differing lengths of supplementation.

CHAPTER II

REVIEW OF LITERATURE

Aspects of Meat Tenderness

During the summer of 1991, the beef industry embarked on a study to evaluate the quality of beef production in the United States. The focus of this effort was to establish baselines for present quality shortfalls and to identify targets for desired quality levels by the year 2001. The 1992 National Beef Quality Audit (Smith et al., 1992) and the 1994 National Beef Tenderness Conference have clearly demonstrated that excess fat and the inability to routinely attain tender meat are the major quality problems facing the beef industry.

A total quality management philosophy to beef production has received much attention in the popular press. While total quality management has primarily focused on production, a new approach to the beef industry is to integrate at each production interface. In other words, all production sectors must identify their quality shortcomings to increase the value of the product at each segment of the production scheme. This concept can be applied to improving beef palatability. Palatability Assurance Critical Control Points identify several antemortem and postmortem sources

for variations in beef tenderness (Smith et al., 1992). A tremendous amount of research documents that the breed of cattle and manor in which these cattle are fed contribute greatly to the variability that exists in our industry. However, a much underutilized opportunity to influence meat tenderness exists at the cellular level. Understanding the interrelationships of the highly dynamic nature of early postmortem events will determine the extent to which we can improve the consistency of meat tenderness. Therefore, it is important to identify the structural and biochemical entities of postmortem muscle in efforts to contribute to the total quality management concept.

Skeletal Muscle Structure

A review of skeletal muscle structure and its constituent myofibrillar proteins is important for an understanding of the action of ethanol on beef cattle performance and meat tenderness. This treatise, unless otherwise noted, will focus on mammalian skeletal muscle.

Skeletal muscle fibers are held in an anatomical pattern by a series of continuous connective tissue sheaths. An entire muscle is surrounded by a connective tissue sheath called the epimysium. Anastomosing from the epimysium is the perimysium which surrounds muscle bundles. Branching from the perimysium is the endomysium which surrounds single muscle fibers. The cell membrane of the skeletal muscle fiber, the sarcolemma, is

closely associated with, but separate from, the endomysium (Romans, et al., 1985).

Skeletal muscle fibers are long, cylindrical, unbranching cells with tapering ends. Each muscle fiber is multinucleated, formed by the fusion of mononucleated myoblasts during embryonic development. The length of a muscle fiber normally ranges from 1 to 40 mm with an average length of 20 to 30 mm. Muscle fiber diameters characteristically range from 10 to 100 μm (Huxley, 1972). Myofibrils are highly organized structures that serve as the contractile components of the muscle cell and comprise about 50% of total muscle protein. Myofibrils are elongated protein threads, 1-3 μm in diameter, lying parallel to one another with the long axis parallel to the long axis of the muscle fiber. Each myofibril extends along the entire length of the muscle fiber (Goll et al., 1984).

Huxley (1958, 1972) reported that myofibrils give rise to the characteristic striated appearance of skeletal muscle when viewed with the phase contrast light microscope. The striated appearance is caused by the alternating patterns of the precisely aligned light and dark bands of adjacent myofibrils. The light regions in the phase contrast light microscope are weakly birefringent (isotropic), and these regions are termed the I-bands. Bisecting the I-band is a narrow, dark line called the Z-line. The dark bands of the myofibril are strongly birefringent (anisotropic), and are called the A-bands. A less dense zone of protein exists at the center of each A-band and

is known as the H-zone. A narrow, dense band of protein, the M-line, is observed with electron microscopy in the center of the H-zone. The distance from one Z-line to the next Z-line is called a sarcomere and is the repeating structural unit of the myofibril. The sarcomere length for resting muscle is 2.3 to 2.8 μm and 1.8 to 2.0 μm for contracting muscle.

Huxley (1972) viewed myofibrils with the electron microscope and described them as being composed of interdigitating thick and thin filaments. The thin filaments comprise the I-band and are 6 to 8 nm in diameter and 1.0 μm long. One end of the thin filament attaches to the Z-line while the other end extends into the region of the A-band between adjacent thick filaments. Thick filaments that compose the A-band region are 14 to 16 nm in diameter and 1.5 μm long. Unlike the I-band, the thick filaments do not attach to the Z-line. Sarcomere lengths change during muscle contraction and relaxation, but the lengths of the thick and thin filaments do not change (Huxley, 1972).

Goll et al. (1984) reported that the proximate composition of mature skeletal muscle is as follows: water, 55-78%; protein, 12-22%; lipid, 2-20%; carbohydrate 1-2%; ash 1-2% and nucleic acid (RNA 100mg/100g; DNA, 25-30mg/100g). The approximate concentrations of other important compounds in living muscle are as follows: ATP, 5-15 mM; ADP, 0.5 mM; phosphocreatine, 20 mM; and creatine, 4-5 mM.

Skeletal muscle proteins have been classified into three groups according to their differing solubilities in salt solutions of various ionic

strengths: sarcoplasmic, stromal and myofibrillar proteins (Szent-Gyorgi, 1960; Goll et al., 1984). Sarcoplasmic proteins make up 30-35% of the total muscle proteins and are soluble in neutral salt solutions having ionic strength of less than 0.2. This fraction contains myoglobin and many of the enzymes associated with carbohydrate, lipid and amino acid metabolism. The stromal fraction is composed of those proteins that are insoluble in neutral aqueous solutions and contains primarily the connective tissue proteins collagen and elastin. This fraction comprises 10 to 15% of the total muscle protein (Goll et al., 1984).

Myofibrillar proteins constitute 50-56% of the total muscle protein and are the largest class of muscle proteins. Generally, myofibrillar proteins are soluble in salt solutions having ionic strengths above 0.4. However, after being removed from the myofibril, some myofibrillar proteins are soluble in water. Thus, it is more appropriate to classify myofibrillar proteins as simply the proteins of the myofibril (Goll et al., 1984). A list of the more important myofibrillar proteins and their respective content as a percent of total myofibrillar protein is as follows: myosin, 45%; actin 20%; tropomyosin, 5%; troponin, 5%; titin, 10%; nebullin, 5%; α-actinin, 2%; B-actinin, 1%; C-protein, 2%; M-protein (165 K protein), 3%); myomesin (185 K protein), <1%; creative kinase, <1%; desmin, <1%; filamin, <1%; and viniculin, <1% (Yates and Greaser, 1983).

Myofibrillar Proteins

Myosin is the primary component of the thick filament (Hanson and Huxley, 1955) and serves an important role in the biological functioning of muscle and in meat quality. Myosin is a very large myofibrillar protein with a molecular weight of about 470,000 and has six subunits. The six subunits consist of two heavy chains of approximately 200,000 to 223,000 daltons each and four light chains of about 16,000 to 21,000 daltons each (Yates and Greaser, 1983). The native myosin molecule consists of a long, double-stranded, almost totally α-helical, rod like tail connected to two globular heads which are the sites of enzymatic activity. Myosin has ATPase enzymatic activity and the ability to bind actin. The globular heads of the myosin comprise the crossbridges which are arranged in a helical fashion throughout the majority of the thick filament. The size and functionability of myosin make it very important with respect to the meat quality factors of tenderness and water holding capacity (Lawrie, 1983).

Actin is the second most abundant myofibrillar protein and comprises 20% of the total myofibrillar protein (Yates and Greaser, 1983). Globular or G-actin is a single polypeptide chain with a molecular weight of approximately 42,000. One G-actin molecule contains one ATP molecule and one Ca^{2+} ion. In the presence of 0.1 M KCl or 1 mM Mg^{2+} , G-actin

polymerizes in vitro to form the double helical fibrous or F-actin. F-actin has the ability to bind myosin and to modify the Mg^{2+}- ATPase activity of myosin. Also, actin forms the backbone of thin filaments and interacts with tropomyosin and troponin. The Ca^{2+}-dependent interaction of actin and myosin in skeletal muscle is controlled by the tropomyosin/troponin regulatory complex (Goll et al., 1984).

Tropomyosin is a long, rod-shaped protein that constitutes 5% of the myofibrillar proteins and is part of the regulatory system of contraction. It is located in each of two grooves of the F-actin helix, associating end to end forming two strands of tropomyosin running the entire length of the thin filament (Goll at al., 1984). The tropomyosin molecule is composed of two polypeptide chains, α-tropomyosin and B-tropomyosin. These subunits are almost entirely α-helical and lie in register in a two-stranded, coiled-coil arrangement (Mak et al., 1980). Each tropomyosin binds one troponin complex.

Troponin, a globular protein with a molecular weight of 69,000 makes up 5% of the total myofibrillar protein fraction. Together, troponin and tropomyosin are responsible for the regulation of muscle contraction via Ca^{2+} specific sites. As determined by SDS-PAGE, native troponin consists of three subunits: troponin T (TN-T), troponin I (TN-I) and troponin C (TN-C) which have molecular weights of approximately 37,000, 24,000 and 18,000 respectively (Goll et al., 1984). TN-T attaches the troponin proteins to

tropomyosin. TN-I inhibits actomyosin ATPase by inhibiting the interaction of actin and myosin. TN-C is capable of reversibly binding Ca^{2+} ions. Huxley (1972) proposed that the troponin-tropomyosin complex controls contraction via a steric blocking model. In the model, when the Ca^{2+} concentration is below 10^{-8} M, tropomyosin is located between actin in the thin filament and the myosin crossbridge. As the calcium concentration rises after its release from the sarcoplasmic reticulum, TN-C binds the Ca^{2+} ions. This binding initiates a series of conformational changes in the thin filament which ultimately allows tropomyosin to move deeper into the actin groove, presumably exposing the myosin binding site on actin. This series of events allows myosin crossbridges to interact with actin in the thin filament, thereby activating contraction. If the muscle dies and there is no ATP available, myosin and actin firmly link to form a rigor complex.

Postmortem Events

After an animal is slaughtered, skeletal muscles undergo a number of biological changes that contribute to the ultimate physical and chemical properties of meat. The magnitude of these changes depends on the species, the environmental conditions and the postmortem handling of musculature. Our knowledge of postmortem events is based on the

pioneering studies of rabbit psoas muscle by Bate-Smith and Bendall (1947; 1949) and Bendall (1951). Of the many processes that are either triggered or arrested by death, only the cessation of blood flow is needed to initiate a cascade of events culminating in rigor mortis. The two consequences of the stoppage of blood circulation is oxidative metabolism ceases and metabolic waste products accumulate. Those processes that are not oxygen dependent continue, thus the potential for biochemical change to occur remains several hours after exsanguination. With the exhaustion of oxygen, glycogen continues to break down, however, because of the oxidative nature of these events, pyruvate is converted to lactate. Lactate is the component responsible for a postmortem pH decline to approximately 5.5 after rigor onset. The rate and extent of this pH decline are highly variable. The development of acidic conditions in early postmortem muscle can have an adverse affect on meat quality.

The degradation of ATP is always accompanied by the regeneration of ATP. The anaerobic phase of glycolysis produces significant, but quite small amounts of ATP. Since oxygen has been depleted from the system, the transition from aerobic to anaerobic glycolysis causes the rate of ATP regeneration to decline considerably. The delay of ATP disappearance is due to the presence of creative phosphate (CP) and the enzyme creatine phosphokinase (CPK) (Bendall, 1973). Creatine phosphokinase promotes the

rephosphorylation of ADP, thus contributing to the maintenance of the ATP reservoir and the accumulation of creatine (C) in the pre-rigor muscle.

$$ADP + CP \rightarrow ATP + C$$

Additionally, a second resynthesizing mechanism assists to a lesser extent. Myokinase rephosphorylates one molecule of ADP at the irreversible expense of another.

$$2\ ADP \rightarrow ATP + AMP$$

During the first few hours of postmortem, creatine phosphate reserves decline to zero, thus, the ATP content of the musculature commences its rapid fall. In pre-rigor muscle, it is the presence of ATP which prevents myosin crossbridges attaching to actin filaments. Change in muscle extensibility precisely parallels the fall of ATP (Bendall, 1973) in the system such that progressive crossbridging occurs between thick and thin filament. Filament sliding becomes increasingly difficult such that muscle extensibility is 5-10% of the tissues' pre-rigor capabilities. When the crossbridge cycle can not continue, rigor is established.

Much variability exists between and within species during rigor onset (Bendall, 1973). Factors affecting this variation include breed, time on feed,

plane of nutrition, electrical stimulation, fiber type, sarcomere length, endogenous enzymatic activity and postmortem aging. The next section will examine these sources of variation and their relevance to meat tenderness.

Interrelationships of Meat Tenderness

A considerable amount of research has been conducted over the last several decades to identify the factors that are responsible for variation in meat tenderness. As with most biological data, an understanding of the problem of meat tenderness is confounded by the degree of interrelationship between the multitude of factors which affect meat tenderness. Of interest to this dissertation are the effects of breed, time on feed, plane of nutrition, electrical stimulation, fiber type, sarcomere length, endogenous enzymatic activity and postmortem aging.

Breed

Tenderness differences between breeds or breed types have often been observed. Many researchers have reported that as the percentage of *Bos indicus* influence increases, the level of tenderness decreases and/or the

variability in tenderness increases. Carpenter et al. (1955) indicated that introduction of the Brahman breed into the United States resulted in decreased beef tenderness in the domestic beef supply. Additionally, Carpenter et al. (1955) reported that rib roasts from full blood Brahman cattle were least tender, from 1/4 Brahman most tender and from 1/2 and 3/4 Brahman intermediate. However, unaged loin steaks were not significantly different among different percentage Brahman, although a similar trend was apparent. The following researchers have documented decreased beef tenderness as the percentage of *Bos indicus* breeding is increased in cattle: Palmer (1963); Ramsey et al., (1963); Dinius et al., (1976); Peacock et al., (1982); McKeith et al., (1985); Wheeler et al., (1989); Whipple et al., (1990).

It should be noted that some Zebu cattle are of acceptable tenderness (Cover et al., 1957). One-fourth Brahman (1/4 Hereford and 1/2 Angus) were not different in sensory tenderness ratings from Angus X Hereford steers when both were fed to 482 kg (Bidner et al., 1986). However, they also found that shear force was lower for meat from Angus X Hereford cattle, although both groups produced tender meat. Furthermore, a 264 member consumer panel found no difference in tenderness of loin, rib or round steaks between the two breed groups. Their data indicate that one-quarter (1/4) Brahman cattle produced meat acceptable in tenderness, particularly to untrained consumers.

Peterson et al., (1979) found no difference in the shear force values of 2.54 cm cores between three-way cross steers which were 1/2 Charolais versus 1/2 Brahman when fed to a USDA Choice slaughter endpoint (Peterson et al., 1979). McKeith et al. (1985) reported that Brahman cattle produced less tender meat than Angus cattle after 0,112 or 224 days on feed. One-half blood Brahman cattle produced less tender meat than Angus after 112 and 224 days on feed. Longer time on feed improved the tenderness of Brahman and Brahman crosses more than the Angus cattle (McKeith et al., 1985).

Time on Feed

Feeding of cattle to improve meat palatability is a common practice in the United States beef industry. Tatum (1981) reviewed the scientific literature on the question of whether tenderness is nutritionally controlled. He suggests that the primary manner in which feeding improves tenderness is by increasing carcass weight and fatness, thereby reducing the rate of postmortem chilling and cold shortening. Moody (1976) also reviewed this topic and found that, within limits, the effect of the feeding period was minimal. Conversely, Zinn et al., (1970a) found a positive correlation between length of feeding period and meat tenderness. Additionally,

increased time on feed was associated with increased carcass maturity (Zinn et al., 1970a; Tatum et al., 1980). However, Zinn et al. (1970a) reported that it was not until after 180 days on feed that animal age exerted a greater negative influence on tenderness.

May et al. (1992) fed Angus and Hereford steers a high concentrate diet from 0 to 196 days. Steers were sixteen months old at the beginning of the feeding period and were slaughtered every twenty eight days until the end of the feeding trial. Taste panel tenderness, amount of perceived connective tissue and shear force peaks at 112 days were all slightly less desirable for cattle fed longer than 112 days. Miller et al. (1987) fed steers a high energy concentrate for 0, 56, 112 or 168 days. All steers were slaughtered at twenty months of age. Tenderness of longissimus muscle and semimembranosus muscle steaks increased with time on feed; however, the greatest improvement was observed from 0 to 56 days. Zinn et al. (1970b) and Dolezal et al. (1982a) fed cattle from 150 to 270 days and 90 to 200 days respectively, and found that quality grades and marbling scores were highest for longer times on feed. Zinn et al. (1970b) found that the deposition of intramuscular fat was not a continuous process, but it occurred at 60 to 90 day intervals.

No improvement in Warner-Bratzler shear (WBS) values were observed for steaks from longissimus dorsi (LD) muscles due to increased time on feed (Marchello et al., 1967). Tatum et al. (1980) found that WBS values

generally decreased as degree of marbling increased, which resulted from a longer time on feed. However, this was not a linear relationship, nor was there always statistical significance in decreasing WBS force with increasing degree of marbling (Tatum et al., 1980). Matulis et al. (1987) also reported that WBS values of longissimus muscle decreased ($P < .05$) with increased time on feed. Similarly, animals fed 100 days or more had lower ($P < .05$) longissimus muscle WBS values than those animals fed for less than 90 days (Dolezal et al., 1982a). Similar results were also reported by Gutowski et al (1979) who found WBS values were lower ($P < .05$) for long-fed cattle (98d) than for short-fed cattle (48d). When comparing 150, 180, 210, 240 and 270 days on feed, Zinn et al. (1970b) demonstrated that cattle fed 240 and 270 days had the highest WBS values. This indicated that extremely long feeding periods or increased animal age are detrimental to tenderness. Short feeding periods of 7, 21 and 42 days postpartum of once-calved heifers did not greatly influence palatability characteristics (Bond et al., 1986). While some studies have concluded that feeding an energy-rich diet prior to slaughter will improve palatability (Tatum et al., 1980; Dolezal et al., 1982a), others have observed diminutive advantages or negative effects from extensive feeding periods (Zinn et al., 1970a). Tatum et al. (1980) and Dolezal et al. (1982b) concluded that the length of feeding required to obtain the desired flavor, juiciness and tenderness of beef appear to be optimal at approximately 100 days for yearling cattle fed a normal finishing diet.

Plane of Nutrition

The growth rate of cattle has an important effect on tenderness (Aberle et al., 1981). These authors suggested that a relationship between the plane of nutrition and tenderness may be due to amounts or to the activity of endogenous proteolytic enzymes present at time of slaughter. Therefore, the preslaughter growth rate could be a more important indicator of meat tenderness than the length of time that a high energy diet is fed. This would be especially true if the high energy diet has been fed for some minimum period of time to permit maximum activity of all enzyme systems which operate in rapidly growing musculature.

Cattle fed high energy diets grow faster, have higher quality grades, may have increased rates of protein turnover, more soluble collagen, or more myofibrillar fragmentation (Aberle et al., 1981; Wu et al., 1981; Hall and Hunt, 1982; Miller et al., 1983). Fishell et al. (1985) reported that when steers were fed a high, medium or low energy diet to obtain an average daily gain (ADG) of 1.42, 0.77 and 0.34 kg per day, sensory panel tenderness scores were highest ($P < .05$) for the high ADG steers. However, there were no significant differences in longissimus muscle WBS values for different rates of ADG, although the high ADG steers tended to have lower WBS values (Fishell et al., 1985). Several researchers have reported that increased growth rate before slaughter is accompanied by increased collagen

synthesis and turnover (Wu et al., 1981; Miller et al., 1983; Fishell et al., 1985). Bailey et al (1974) reported that as growth rate decreases, collagen cross-links stabilize and the more stabilized cross-links result in less tender meat. Therefore, it is not the amount of collagen present, but rather the quality of the collagen that contributes to meat texture (Bailey et al., 1974). Hall and Hunt (1982) reported that steers slaughtered while in the A-maturity age range, which had tolerate wide ranges in feeding regimen, had little affect on tenderness, amounts of total collagen or collagen solubility. Accelerated production in which steers are fed a high energy diet beginning after weaning and slaughtered at a USDA Good endpoint, resulted in longissimus muscle palatability at least equal to palatability of conventionally produced steers (Dikeman et al., 1985).

Electrical Stimulation

Electrical stimulation is effective in improving beef tenderness when applied during slaughter. The first documented use of electrical stimulation for tenderizing meat came when Benjamin Franklin reported in 1749 that killing turkeys electrically made them uncommonly tender (Lopez and Herbert, 1975). Application of electricity to pre-rigor muscle has been shown to accelerate glycolysis (Carse, 1973; Chrystall and Devine, 1978; Clarke et al.,

1980; Forrest and Briskey, 1967), thereby increasing the rate of pH decline (Bendall et al., 1976; Bouton et al., 1978; Devine et al., 1979; Hallund and Bendall, 1965). Electrical stimulation produces a high temperature - low pH condition within the muscle; a condition which could rupture the lysosomal membrane and which is conducive to enzyme activity (Dutson et al., 1980; Moeller et al., 1976; Sorinmade et al., 1978). As a result, steaks obtained from electrically stimulated carcasses may have a decreased aging requirement (Savell et al., 1978) and can have enhanced tenderness, flavor and overall palatability (Cross et al., 1979; Davey et al., 1976; Riley et al., 1980; Smith et al., 1979).

New Zealand investigators (Chrystall and Hagyard, 1975) researched the use of electrical stimulation to accelerate conditioning of lambs, and thereby prevent cold toughening of carcasses chilled or frozen prerigor. They concluded that the prevention of cold shortening accounted for the improvement in tenderness. Cross (1979), however, concluded that cold shortening alone did not explain the differences in tenderness due to electrical stimulation. Other proposed mechanisms for the tenderizing effect of electrical stimulation include: enhanced enzyme activities (Sorinmade et al., 1978; Dutson et al., 1979) and physical disruption of muscle fibers from stimulation - induced contractions (Savell et al., 1978).

Fiber Type

The characteristics of muscles are a reflection of the proportions of fiber types present. Needham (1926) reported muscle was a heterogeneous mixture of red and white myofibers. There are distinct differences in the enzymatic activity, metabolism and contractile properties of the different fibers that constitute striated muscles. Type I fibers are also know as slow twitch oxidative or red fibers. They have low actomyosin ATPase activity and are of moderate diameter. Their glycolytic capacity is moderate, however, the oxidative capacity is high. Type IIB fibers are also termed fast glycolytic or white fibers. Their actomyosin ATPase activity is very high, with high glycolytic and low oxidative capacity. With respect to fiber diameter, they are considered to be large. Type IIA fibers are a combination of the other two fiber types: fast oxidative with high actomyosin ATPase activity, small diameters, high glycolytic and oxidative capacities and are also named intermediate fibers.

There are differences between Type I and IIB fibers with respect to enzymatic activity, amino acids uptake and protein and RNA content. Type I fibers react slowly and have a long latency period which are adapted for long, slow contractions (Goldberg, 1967). Type IIB fibers have short twitch intervals and are specialized for fine, skilled movement and fast contractions. Type IIB fibers have greater concentrations of protein in the sarcoplasm than

type I fibers, yet myofibrillar concentrations in the two types of fibers are similar (Barany et al., 1965). Muscles with predominantly red fibers have a greater RNA content, accumulate amino acids at a faster rate and have a faster rate of protein turnover (Goldberg, 1967; Li and Goldberg, 1976).

During the first year of growth for steers and heifers, the mean cross-sectional area of red, white and intermediate fibers doubles, the percentage of red fibers decrease while the percentage of white fibers increase. Heifers have larger muscle fibers than steers (Spindler et al., 1980). Bulls have a higher ($P < .05$) percentage of red fibers and a lower ($P < .01$) percentage of white fibers than steers, additionally, bulls also have larger mean fiber diameters than steers (Okerman et al., 1984; Seideman and Crouse, 1986).

Difference in the diameter and percentage of fiber types have been suggested as affecting meat tenderness (Calkins et al., 1981; Ockerman et al., 1984; Seideman and Crouse, 1986). Moody et al. (1970) found that fiber diameter was not related to tenderness. Similar results were reported by Whipple et al. (1990) and Romans et al. (1965). Melton et al., (1975) reported that red fiber area was related to hot carcass weight, fat thickness and cutability, but not to palatability traits. Research by Tuma et al. (1962) and Crouse et al. (1991) suggests that fiber diameter can directly affect tenderness prior to postmortem aging.

Further work by Crouse et al. (1991) reported that the percent of type IIB fiber was positively correlated ($r = .60$) to shear force when steaks were

aged for twenty four (24) hours; however, when steaks were aged between 3 and 14 d, this correlation was not significant. Ashmore et al. (1972) measured the percentage of fiber types across a wide range of carcass maturities. When averaged across carcass maturities from A to E, shear force was positively correlated ($r = .35$) to the percentage of type IIB fibers but negatively correlated ($r = -.25$) to the percentage of type IIA fibers. Therefore red fibers appear to be more indicative of increased tenderness. Psoas major, considered to be predominantly red fibers, was studied by Lewis et al. (1977). Lewis and coworkers found a significant correlation ($r = .37$; $P < .01$) between percent fiber area and tenderness when evaluating psoas major. They also reported that even though breed had an effect on fiber diameter, breed had little effect on tenderness. It was therefore recommended by Lewis et al. (1977) that cattle selected for larger fiber diameters and increased muscling would not affect tenderness as long as aging times were sufficient. British and dairy breeds have higher proportions of red fibers relative to continental breeds. There appears to be a positive correlation between the percentage red fibers and intramuscular fat. Therefore, fiber type may be indicative of eating quality parameters.

Sarcomere Length

Several researchers have studied the effects of sarcomere length on meat tenderness. Gothard et al. (1966) stated that sarcomere length is significantly related to tenderness, but cautioned that this was not necessarily the most responsible factor influencing tenderness. Bowling et al. (1977) reported that sarcomere lengths differed among meat from cattle produced by three feeding systems, as did shear force values of cooked muscle. Dutson et al. (1976) conducted a study which revealed that the shortening of sarcomeres was detrimental to sensory tenderness scores. Significant correlations were found between shear force values or sensory tenderness ratings and sarcomere length (Cooper et al., 1968). Others have not established an absolute relationship between these traits. Parrish et al. (1973) found an increase in sarcomere length 1d postmortem with very small subsequent changes. Interestingly, tenderness continued to increase beyond the first day. Similarly, Locker and Daines, (1976) showed that increasing the muscle temperature to 37°C during the final stages of rigor negates the toughening effect of cold shortening. They concluded that the temperature during the final stages of rigor played a significant role in tenderness, regardless of sarcomere length. Thus, it would seem that muscle temperature and/or pH plays a primary role in the contractile state of pre-rigor muscle and ultimate meat tenderness. Lochner et al. (1980) stated that

the cooling rate significantly altered meat tenderness despite the lack of any difference in sarcomere lengths between treatments.

Results of several investigations (Moller and Vestergaard, 1987; Feldhusen et al., 1992) indicate that chilling conditions currently used in industry are correlated with large changes in the contractile structures of skeletal muscle. The influence of low temperatures on sarcomere lengths have been reviewed by Locker and Hagyard (1963) and Stromer and Goll (1967). The extent of shortening depends largely on the surrounding temperature. Honikel et al. (1986) reported minimal shortening between 10 - 20°C while 50% shortening occured at temperatures less than 10°C. Lee (1984) demonstrated that a decrease in tenderness results when sarcomere shortening exceeds twenty percent (20%). This change is assumed to be caused by a greater filament density as a result of the overlapping of the myofilaments (Goll et al., 1974). There are differing views on the influence of postmortem aging on sarcomere length. Such contradictory studies include Shorthose et al. (1984) and Moller and Vestergaard (1987). Feldhusen et al. (1992) concluded that aging musculature for 72h resulted in considerable lengthening of sarcomeres. Liebich and Kieslich (1985) discussed the reversibility of the actomyosin rigor state in muscle which had undergone cold shortening. Kuhne (1990) reported distinct evidence for these changes when examining ultrastructural properties. Additionally, considerable lengthening of sarcomeres also occurs when the rate of

glycolysis is accelerated. These changes appear to take place immediately post-rigor as suggested by Feldhusen et al., (1992).

Correlations between sarcomere lengths and tenderness has been reported to be low (Kuhne, 1990). The slight connection between tenderness and sarcomere length may be related to changes in water holding capacity. The combination of temperature and pH appears to affect the ability of early postmortem muscle to contract; however as important as these variables are to postmortem length changes, later stages of rigor development may be more dependent on the conditions that are conducive to enzymatic activity.

Endogenous Enzymatic Activity

An important aspect of tenderness has recently focused on the proteolytic mechanisms of postmortem aging. Numerous changes occur during postmortem storage of carcasses or vacuum-packaged meat that are attributed to the structural degradation of the myofibril by proteolysis. The enzymatic activity of these proteases are affected by a multitude of factors. To simplify, this discussion will concentrate on only two proteolytic systems that are present in skeletal muscle, the calcium dependent proteases and the lysosomal enzyme system. It has been clearly demonstrated that both of

these systems are integral parts of the tenderness equation. Postmortem proteolytic enhancement of tenderness has been well documented by Parrish et al., 1973; Goll et al., 1983; and Koohmaraie et al., 1988).

Calcium Dependent Proteases: Calcium-dependent proteases (CDP) have been referred to by a variety of names in the literature. Such nomenclature includes calcium activated factor, (Busch et al., 1972; Olson et al., 1977; Koohmaraie et al., 1986) calcium-dependent neutral proteases, (Vidalene et al., 1983; Ducasting et al., 1985) calcium-activated protease (Suzuki et al., 1982) and calpain (Murachi, 1985).

With respect to biochemical properties, Meyer (1964) showed that calcium dependent proteases do indeed exist in skeletal muscle. Busch et al., (1972) further characterized the protease and was later purified by Dayton et al., (1976a). As the next twenty years passed a ubiquitous distribution of the CDP system in animal cells was apparent. The classical biochemical analysis by Murachi (1983) had shown that the CDP system was localized mostly in the cytosol. The location for calpain must therefore be the cytosolic, soluble fraction of the cell. Suzuki et al. (1987) clearly demonstrated that CDP can be translocated to the sarcolemma, nuclei (Schollmeyer, 1988) and mitochondria (Beer et al., 1982). Furthermore the association of CDP with a number of cytoskeletal proteins is highly probable since such proteins as actin, alpha-actin, nebulin and titin are quite

susceptible to CDP hydrolysis, (Murachi et al., 1987; Wang, 1982; Wang and Wright, 1988).

The CDP system consists of four primary components; μ-calpain, m-calpain, calpastatin and an activator protein. Micromolar concentrations of calcium is required for activation of μ-calpain while millimolar concentrations are required for m-calpain. Skeletal muscle calpains have a molecular weight of approximately 110 kDa under non-denaturing gel electrophoresis which dissociates into 80 kDa and 28 kDa subunits as shown by SDS-PAGE. The amino acid sequences of both the 80 kDa and 28 kDa subunits of calpains purified from different species are highly homologous. Hence, the calpains seem to be highly conserved molecules.

A number of results show that the activity of μ-calpain and m-calpain is possibly regulated by two different mechanisms. Cong et al. (1989) found that phosphatidylinositol lowers the specific activity of μ-calpain to ten percent of its original activity, but has no effect on the specific activity of m-calpain. However, Rozanov and Mellgren (1988) have recently shown that a number of phospholipids, including phosphatidylserine, phosphatidylethanolamine and phosphatidylcholine, all lower the concentration required for autolysis of m-calpain, although phosphatidylinositol is the most effective. Binding to a membrane-phospholipid has been proposed to lower the concentration for autolysis to occur at physiological conditions of free calcium. The autolyzed enzymes are

then either released into the sarcoplasm which they are now able to hydrolyze their substrate proteins, or they remain associated with the cell membrane and degrade cytoskeletal proteins which interact with the sarcolemma. Activity of the autolyzed calpains are subject to the final regulation by calpastatin.

Calpastatin, an inhibitory protein, decreased the activity of both μ-calpain and m-calpain at the respective calcium concentrations required for catalytic activity. It has been suggested that all cells that contain calpain also contain calpastatin, although the ratio of calpains to calpastatin varies from cell to cell. In general, immunolocalization studies have found that μ-calpain, m-calpain and calpastatin are all widely distributed throughout the cytoplasm in a variety of cells. Early studies by Dayton and Schollmeyer (1981) suggested that m-calpain was localized at the Z-disk in striated muscle cells, however, more recent immunogold studies by Kumamoto et al. (1991) indicate that both μ-calpain and m-calpain and calpastatin are located in all regions of the myofibril and the remainder of the muscle cell. Interestingly, both calpains and calpastatin are approximately two times more prevalent at the Z-disk than in the A- or I- band areas of the myofibril. This higher concentration at the Z-disk may have given some researchers the impression that calpains and calpastatin were located exclusively at the Z-disk (Dayton and Schollmeyer, 1981). Additionally, only a small proportion of the total cellular calpain of calpastatin content is preferentially located

near the sarcolemma, therefore, calpastatin alone cannot account for the regulation of calpain activity in skeletal muscle. Perhaps further understanding of the role of phosphatidylinositol, the 28 kDa subunit or a calpain activator may help to unravel the mechanism of calpain regulation.

Optimal enzymatic activity for μ-calpain is at 21°C and pH 7.0 with minimal activity at 5°C and pH 5.6. The postmortem decrease in muscle temperature and pH reduces μ-calpain activity. As a result, μ-calpain possesses minimal activity under conditions normally associated with postmortem muscle (Calkins and Rhynalds, 1989). Koohmaraie et al. (1987) reported approximately fifty percent of the maximal μ-calpain activity is lost during the first twenty-four (24) hours postmortem, whereas m-calpain activity is unchanged. Numerous factors seem to affect the activities. These include breed (Wheeler et al., 1989; Johnson et al., 1990; Whipple et al., 1990), B-agonists, (Forsberg et al., 1987), anabolic implants (Sinnett-Smith et al., 1983), and glucocorticoids (Griffin and Wildenthal 1978).

Although calpains are extremely substrate specific, it appears that they do not require a specific amino acid sequence but only a long polypeptide (Bandman, 1987). Postmortem aging and calpains have the following effects on myofibrils: Z-disk degradation, myosin degradation, degradation of troponin-T to a 30 kDa component, degradation of desmin, degradation of titin and increases the Mg^{2+} - modified and Ca^{2+} - modified ATPase activity (Goll et al., 1983; Koohmaraie et al., 1986). However, actin, α-actinin and

troponin-C are resistant to calpain (Dayton et al., 1976a, 1976b; Ishiura et al., 1980).

Johnson et al. (1990) found high correlations between μ-calpain and Warner Bratzler shear values after twenty-four (24) hours postmortem. Koohmaraie et al. (1988a) noted the μ-calpain, m-calpain and calpain inhibitor activities in bovine longissimus muscle were twice as high than in psoas major ($P < .05$). It is interesting to note that psoas major had lower shear force values compared to longissimus muscle ($P < .05$).

Wheeler et al. (1989) reported Brahman longissimus muscle had significantly lower μ-calpain and higher calpain inhibitor activity immediately postmortem than did the muscle from Herefords at optimum conditions for μ-calpain activity. Whipple et al. (1990) state the calpain inhibitor as one possible causes for the difference in tenderness between Bos indicus and Bos taurus cattle. Johnson et al. (1990) found no differences between breed crosses for μ-calpain and m-calpain activity, but the activity for calpain inhibitor was significantly greater in 3/4 Brahman than in 1/2 Brahman and Angus carcasses. Shackleford et al. (1991) reported the activity of the calpain-inhibitor was greater in the 5/8 Brahman crosses compared to Angus-Hereford crosses. Ou et al. (1991) found that castration did not alter muscle calpain or calpastatin activities in lambs.

Decreases in μ-calpain activity during postmortem storage indicates that the enzyme is activated and then autolyzed (Vidalenc et al., 1983;

Ducastaing et al., 1985; Koohmaraie et al., 1987). Koohmaraie et al. (1988a) investigated protease activity in three muscles (psoas major, biceps femoris and longissimus dorsi). Higher aging response was associated with high μ-calpain activity (longissimus dorsi) and lower aging response with lower activity (psoas major). Thus, it was concluded that initial levels of μ-calpain activity may determine the aging response of a given muscle. By incubating muscle slices under conditions to either activate or inactivate the calpain system, Koohmaraie et al. (1988b) concluded that postmortem muscle changes were calcium mediated and that μ-calpain had a significant role in these changes. Additional evidence supporting the role of μ-calpain in meat tenderness was demonstrated by infusing lamb and beef carcasses with $CaCl_2$ solution. The $CaCl_2$ treatment resulted in tenderness levels at one day postmortem which were similar to untreated carcasses that were aged seven days. Furthermore, the tenderness enhancement was attributed to increased ionic strength from calcium infusion (Koohmaraie et al., 1988b, 1989). However, samples from NaCl infusion were equal in tenderness at seven days postmortem to those infused with $CaCl_2$ and thus, both $CaCl_2$ and NaCl infusions resulted in meat that was much more tender at seven days postmortem than non-infused meat.

Lysosomal Enzymes: Ono (1970) suggested that since muscles from the same animal differ in tenderness, there was a need to study the lysosomal enzymes of specific muscles. Seven catheptic enzymes have been identified which are released from skeletal muscle lysosomes and have maximal activity when the pH of muscles decreases to a range of 4.0 - 6.0 (Dutson, 1983; Goll et al., 1983). Cathepsins hydrolyze a number of myofibrillar proteins (i.e. myosin, troponin-T, actin, titin, nebullin) and connective tissue proteins (i.e. collagen).

Cathepsin B is a endopeptidase that requires EDTA and reduced SH groups for full activity and is inhibited by thiol regents (Bond and Butler, 1987). It degrades myosin, actin and intact myofibrils (Goll et al., 1983). Cathepsin L, like B, is a thiol protease with endopeptidase activity. Cathepsin L is estimated to have ten times greater specific activity against myosin than cathepsin B (Bird and Carter, 1980). For cathepsin B to degrade myosin and actin, an optimal pH between 3.5 and 6.0 is required (Asghar and Bhatti, 1987). Wheeler et al. (1990) stated that cathepsin B and L activities could be related to postmortem tenderization of meat. Johnson et al. (1990) reported that cathepsin B and L activities in longissimus dorsi muscle was negatively correlated to Warner-Bratzler shear values at day ten postmortem. Calkins et al. (1989) and Johnson et al. (1989) found a significant relationship with postmortem aging in dietarily manipulated and Brahman crossbred cattle respectively. Koohmaraie et al. (1988a, 1988b,

1989) reported that cathepsin activity was not related to postmortem tenderization.

Perhaps the most important cathepsin affecting meat tenderness is cathepsin D. Several investigators have shown cathepsin D to be more active in proteolysis than cathepsin C (Huang and Tappel, 1971), cathepsin B (Morrison et al., 1973; Schwartz and Bird, 1977) and cathepsin A (Iodice et al., 1966). Careful studies of cathepsin D preparations indicate that it has activity on myosin and actin (Dingle et al., 1971; Robbins et al., 1979). Co-incubation of cathepsins B and D does not result in synergism with the end products resembling those of cathepsin D. When the end products of cathepsin B were incubated with cathepsin D, they were further degraded, but no additional effects were seen on the end products of cathepsin D. Regardless of the relative importance of the different cathepsins, it is clear that cathepsins exert proteolytic activity on muscle proteins and that cathepsins may be involved in postmortem tenderization.

Postmortem Aging

Aging or conditioning of meat is the process of storing meat for varying periods of time under controlled temperature and relative humidity. From a commercial viewpoint, the aging of meat is a most important process

as it becomes appreciably more tender. Industry storage criteria is typically 0-3 °C for 7-14 days, however, the logistics of inventory and storage facilities may dicatate otherwise. Morgan et al. (1991) found that the average post-fabrication time, defined as the time for primals and sub-primals to arrive to retail outlets, for all cuts was approximately 17d. Minimun and maximum post-fabrication times ranged between 3 and 90 days. It should be clearified that the freezing for sub-primals increases post-fabrication time; however, the rate of change to post-rigor musculature attributible to aging decreases greatly.

Enhancing tenderness during postmortem storage has been studied extensively. This postmortem tenderization has been related to structural changes in the myofibril, to changes in the myofibrillar protein compostition and to the action of endogenous proteolytic enzymes (Penny and Dransfield, 1979; Penny 1980; Yates et al., 1983; Weber 1984).

Minimal structural changes to the conective tissue frame work have been documented. McClain et al. (1970) found that the yield of intramuscular connective tissue form beef was less at 3d postmortem than at time of slaughter. Pfieffer et al. (1972) detected small changes in the covalent cross-linking of connective tissue after 21d of storage of beef muscle.

The progessive disappearance of the Z-disk was intially reported in 1967 (Davey and Gilbert, 1967; Fukazawa and Yasui. 1976). After 2 or 3

days of aging at temperatures between 0 and 4°C, the Z-disk is less distinct and small lateral breaks within the structure are commonly seen, thus providing evidence of a weakened Z-disk structure (Davey and Dickson, 1970). The rate of degradation of Z-disks and the appearance of gaps in the I-Z junction is not the same for all fiber types. Z-disks of type IIB fibers degrade at a faster rate than type I fibers whose Z-disks are nearly twice the thickness of type IIB (Gann and Merkel, 1978).

Comprising the well ordered structure of the muscle cell is myofibrillar proteins. It appears that α-actinin is the most likely component of the Z-disk to undergo change. In accordance with Chin-Sheng and Parrish (1978), the bonding of α-actinin to the Z-disk weakens during aging. Assuming that α-actinin plays a key structural role in anchoring opposing thin filaments from adjacent sarcomeres, its degradation must lead to a considerable decrease in myofibrillar tensile strength.

The troponin complex lies in the thin filaments of myofibrils and is involved in the regulation of muscle contraction. SDS-PAGE results revealed that myofobrils of aged meat have a gradual disappear of troponin-T (Penny and Dransfield, 1979; Olson et al., 1971; Yamamoto et al., 1979). This degradation of troponin-T is also considered an indicator of proteolysis (MacBride and Parrish, 1977; Parrish and Cheng, 1979; Yates et al., 1983). Many researchers have shown the appearance of a 30 kDa component whose concentration increases during aging (MacBride and Parrish, 1977;

Valin et al., 1981; Ouali, 1983). By measuring the intensity of the troponin-T band in gels by densitometry, Penny and Dransfield (1979) demonstrated that the reduction in toughness during aging was quantitatively related to the loss of troponin-T. The rate of loss of troponin-T paralleled a reduction in toughness when measured over a range of storage temperatures from 3°C to 35°C. The correlation between the extent of loss of troponin-T and toughness was 0.78. The study of troponin-T has made an important contribution to the understanding of postmortem myofibrillar changes because it's degradation provided the first evidence that structural changes are related to meat tenderness and do indeed occur during aging.

Appreciable interest in recent years exists in determining the effects of aging on the cytoskeletal framework of muscle cells. Pronounced quantitative changes occur to the cytoskeletal protein desmin. Based on SDS-PAGE of guanidine extracts, desmin progressively disappears during the aging of muscle and is presumed to be destroyed by proteolysis (Young et al., 1980). Titin, another cytoskeletal protein, accounts for 6% of the myofibrillar bulk in muscle cells (Greaser et al., 1981). Rapid proteolytic degradation of titin has been shown to occur during meat heating at 55 °C (King et al., 1981). Davey and Gilbert (1976) found that the maximal aging rate occurs at 55° to 60°C; therefore suggesting that the relationship of cytoskeletal protein degradation and meat tenderness is temperature related.

Lochner et al. (1980) found a correlation of 0.8 between loin tenderness and the temperature attained by the longissimus muscle at 2-4 hours postmortem. In a later study, Marsh et al. (1981) exposed carcass sides to 37°C until 3 hours postmortem, after which normal chilling was applied. This treatment brought about a significant improvement in tenderness. The rate of tenderness enhancement was far higher for the "hotbox" treatment when compared to controls (normal chill only). It was concluded that the rate of tenderness improvement during postmortem aging is affected by a combination of high pH and high temperature. If we were to couple this data with recent proteolytic studies, there is an enormous incentive to pursue the dynamics of early postmortem muscle with renewed vigor.

Effect of Cookery on Meat Palatability

Meat cookery and the effect of heating on specific muscle components is very important, yet has not been as thoroughly researched as many areas which have far less impact upon overall palatability. Several investigations have been conducted to improve the palatability of beef by imposing treatments to enhance preslaughter management, nutrition, postmortem aging/handling, and evaluating effects of gender, physiological maturty,

connective tissue characteristics, myofibrillar fragmentatin, and proximate compostition of muscle. As consumer preferences have moved towards reducing caloric intake and limiting the ingestion of saturated fats, researchers have investigated research approaches to produce meat products which satisfy these criteria. However, of particular importance, is evaluating how cookery can optimize desirable palatability attributes.

Flavor

Nearly all meat is heated prior to consumption. Historically this was for its pasteurization effects, although applying heat invokes characteristic flavor, aroma, texture and appearance attributes to which consumers have become accustomed. Hamm and Hofmann (1965) reported that charcteristic flavors develop at temperatures exceeding 70 °C when oxidation of -SH to -SS groups from actomyosin occurs. Furthermore, during longer heating periods at higher timperatures, H_2S is formed from free or easily reacting groups of actomyosin.

Flavor changes during heating of meat are influenced by the type of cookery and amount of heat applied to the system. Methods of cookery using dry heat, (broiling, roasting) expose surface tissues to high temperatures which result in fluids progessively migrating to the surface.

This acts to concentrate both water-soluble and lipid-soluble flavor components of the species on the surface (Paul, 1972). Furthermore, the texture of fat softens or liquifies. While some fat is lost, some of the fat coats the surface and form aromatic compounds which are volatilized and provide materials for pyrolysis and /or interactions for all the components (Paul, 1972; Allen and Foegeding, 1981). Cookery methods utilizing moisture such as braising provide more optimal conditions for water-soluble flavor compounds to migrate from the meat into the broth or drippings (Paul, 1972).

Cooked meat flavor is also dependent upon internal temperature. Spanier et al., (1990) suggests that hydrolytic enzymes such as lipases, glycosidases and proteinases undergo temperature induced changes which make them excellent effectors for the formation of meat flavor components. The proteinases show various activity levels at different cooking temperatures (Spanier et al., 1990) and can produce several reactive products, including flavor peptides (Yamasaki and Maekawa, 1978; Asao et al., 1987). Furthermore, proteinases are capable of generating a pool of amino acids for the formation of Maillard reaction products (Bailey, 1988). Spanier et al. (1990) reported that the thiobarbituric acid reactive substance (TBA) level of beef semimembranosus muscles oven roated at 177 °C to 51.6, 60.0, 68.3 or 76.7 °C was dependant on end point temperature. TBA levels increased with increasing internal temperature up to 68.3 °C, above

this temperature, TBA levels declined. Gas chromatography analysis revealed that hexanal increased to a maximum by 60°C and then steadily declined with increasing temperature. The drop in TBA and hexanal levels may be explained by an increase in the formation of Maillard reaction products.

Tenderness

In addition to characteristic flavors which evolve during heating, the texture of the meat is also influenced to a large extent. In beef, tenderness may well be the single most important palatability attribute determining counsumer acceptance.

A large tenderness survey was recently conducted by researches at Texas A&M University (Morgan et al. 1991) which suggested that slightly over 20% of the rib and loin cuts and over 40% of the round and chuck cuts would be rated "slightly tough" or tougher as based on Warner-Bratzler shear values related to sensory panel tenderness. Indeed, this is a problem. As one considers the genetic base of today's cattle, our management and production systems and various packing and processing procedures, it is clear that there are no simple solutions to enhancing the tenderness of beef unless mechanical or enzyme induced tenderness systems are applied.

However, these may be met with various concerns over food safety, purge, etc. Therefore, a key aspect of tenderness which seemingly has been somewhat overlooked is that of cookery. Can cookery optimize tenderness in certain cuts of beef that vary in connective tissue and fat compostion?

Previous research by Davey and Gilbert (1974) and Bouton and Harris (1981) indicated that when meat is heated, two distinctly separate phases of toughening occur at different temperatures. In the first phase, a three to fourfold toughening occurs between 40°C and 50°C, believed to be due to the denaturation of the contractile system. The second phase occurs between 65° C and 70°C which results in a doubling of toughness. This second phase of toughening is believed to be mediated by collagen shrinkage and weight loss from the application of heat. Collectively, this data coincides with an increase in shear force.

Several studies have correlated the effect of final internal temperature to the tenderness of meat. Independent studies by Draudt (1972) and Parrish et al. (1973) have shown that degree of doneness had a greater influence on tenderness and overall palatability than did maturity or marbling, In fact, Parrish et al. , (1973a) was first to report no interaction with degree of marbling and internal cook temperature for steak palatability. Furthermore, as internal temperature is increased, the meat becomes harder, drier and less tender as observed by sensory panel scores and shear force

values (Cover et al., 1962; Ritchey and Hostetler, 1965; Parrish et al., 1973a; Hostetler et al., 1976; Cross et al., 1976; Bowers et al., 1987).

Juiciness and Water Holding Capacity

Juiciness is a commonly appreciated texture. It wets and refreshes the mouth and its absence violates expectations and lowers the perceived quality. High quality meats are expected to be juicy (Szczesniak, 1991). In a recent study, Szczesniak and Ilker, (1988) described juiciness as a multi-dimensional perception that includes force with which the juices squirts out, total amount of juice released on chewing, flow properties of the expressed liquid and the contrast in consistency between the liquid and suspended cell debris. Absence of juiciness means that the product is dry and thus, will probably draw moisture from the mouth instead of supplying it to the mouth. Juiciness is a palatability attribute which may closely linked to tenderness. It is influenced to a large extent by the degree of doneness.

Laakonen et al. (1970) reported that degree of doneness in meat is extremely critical in affecting tenderness and cooking yield. They found that if the final internal temperature is below that of the collagen shrinkage temperature (60°C-65°C), then the major reduction in tenderness does not occur. Conversely if the final temperature is greater than that of the collagen

shrinkage temperature, the coagulation results in a higher cook loss percentage and a more tightly packed, less tender muscle (Laakonen et al. 1970). Several researchers have reported that as final internal temperature increases, juiciness decreases (Ritchey and Hostetler, 1965; Parrish et al., 1973a; Cross et al., 1976; Bowers et al., 1987).

Cooking meat causes protein denaturation followed by coagulation of myofibrillar proteins, shrinkage of myofilaments and tightening of the microstructutre of myofibrils (Cheng and Parrish, 1979). Since lean cuts of fresh beef contain approximatley 75 percent water with protein being the principle component responsible for the binding of the water, cookery plays an important role in maintaining maximum cooking yield. According to Davey and Gilbert (1974), the shrinkage of collagen caused by heating above 60°C caused a contracture of the collagen sheath and the exudation of moisture from the myofibrils.

When considering the meat's inherent ability to bind water, only 4-5 percent is tightly bound to actin or myosin, depending on the shape or charge of the protein (Hamm, 1969). Since this represents a small fraction of the total water, it is of less impotance when compared to the fraction immobilized within the muscle tissues which is influenced by the spacial molecular arrangement of the myofibrillar proteins (Hamm, 1969). Tightening the spacial network of the myofibrillar proteins by heating decreases the amount of immobilized water. Therefore, the amount of

expressible moisture increases (Hamm, 1969; Hostetler and Landmann, 1968).

It appears that moisture loss in cooked meat contributes to decrease tenderness. Although this coincides with events related to myofibrillar denaturation and collagen shrinkage, juiciness and tenderness are closely related.

Mechanical Measures of Meat Tenderness

In addition to subjective assessment of meat palatability by sensory panelists, instrumantion has been developed to objectively characterize certain palatability attributes. Various types of instruments which include penetrometers, masicometers, extruders and shear devices have been used to determine meat tenderness attributes.

Pentrometers are based on the principle of inserting a probe or needle into the tissue and measuring the force required for a given penetration depth. These systems have a strain-guage associated with each probe and the force reading is dependant upon the resistance of the tissue. The Armour Tenderometer is such a device. It was hoped that this system could accurately segregate beef carcasses into tenderness groups whereby carcasses could then be marketed accordingly. However, it was not shown

to be a reliable indicator of tenderness (Carpenter et al., 1972; Dikeman et al., 1972; Henrickson et al., 1972; Parrish et al., 1973b).

Various masicometers, extruders and cutting devices have been used to determine tenderness of muscle and meat (Szczesniak and Torgeson, 1965; Szczesniak, 1973). These have not been extensively used in meat science textural studies, particularly when compared with devices that measures shear force.

The Warner-Bratzler shear (WBS) device is the best known apparatus which meat scientists use to objectively and quantitatively assess meat tenderness. This device is composed of a 1mm thick metal blade which has a triangular opening into which a cylindrical meat sample core, usually either 1.27 or 2.54 cm in diameter, is inserted (Bratzler, 1949). Shear bars on each side of the triangular blade are driven downward through the longitudinal axis of the muscle fibers in the core which separates it into two pieces. The maximum force exerted on the blade is detected by a dynameter spring and read from a dial scale or from a printout if using a WBS test cell fitted for the Instron Universal Testing Machine.

Bouton et al. (1975) and Seideman and Theer (1986) investigated shear force value and certain relationships associated with tenderness. When Bouton et al. (1975) analyzed shear force deformation curves, they found that the curve containd two segments. They reported that the first curve, intial yield force required to compress and initiate shear fracture

planes through the myofibrils, was primarily influenced by myofibril strength. The second portion of the curve, the difference between tht initial yield force and the peak force, could be an indication of the strength of the connective tissue. Furthermore, these investigators found that initial yield force values: 1) increased with internal temperature as degree of doness exceeded 60 °C; 2) decreased with advancing postmortem storage interval; 3) was not significantly influenced by animal age. In addition, Bouton et al. (1975) reported the difference between initial yield and peak force was: 1) not influenced by postmortem aging of carcasses form young or old animals; 2) significantly increased with animal age; 3) significantly reduced by cooking meat to a 90 °C internal temperature.

It has been shown that high correlations between sensory panel tenderness and WBS values exist. Previously reported correlations between sensory panel tenderness scores and WBS includes: r=-0.62. Huffman, 1974; Olson and Parrish, 1977; Crouse et al., 1978); r=-0.63 (Goll et al., 1965); r=-0.65 (Moe et al., 1964); r=-0.75 (Field et al., 1966); r=-0.78 (McBee and Wiles, 1967); r=-0.88(MacBride and Parrish, 1977); and r=-0.90 (Culler et al., 1978). Certain studies, however, have found poor correlations between sensory panel tenderness and WBS value. Breidenstein et al. (1968) reported a correlation of r=-0.33 and Parrish et al. (1973b) reported a correlation of r=0.30 for sensory panel tenderness and WBS value.

Part of the reason for the variation observed between WBS value and sensory panel tenderness may be due to the lack of homogeneity of the meat tissue. Shear tests have been used by many researchers who have evaluated core position effects in beef muscles. Alsmeyer et al. (1965) and Tuma et al. (1962) found greater tenderness in the medial region of the beef longissimus. Furthermore, cores from the dorsal region were more tender than those from the central or lateral region in longissimus muscle roasts Williams et al. (1983). However, Smith et al. (1969) found the greatest tenderness in beef steak was in the central region . Although, Sharrah et al. (1965) and Cover et al. (1962) reported that the lateral region was the most tender. These last two studies are in agreement with results from a recently published study by Crouse and coworkers (1989), who reported tenderness of cores improved from dorsal to lateral. Therefore, location effects of tenderness within the longissimus muscle could be useful in future studies of the physical/biochemical effectors of tenderness.

Various research institutions, in attempts to minimize expenses (time and financial), have elected to simply obtain WBS values and dispense with sensory panel research. However, tenderness is perhaps the most important palatability attribute. Since near perfect correlations between sensory panel tenderness and WBS values do not exist, and experienced meat sensory researchers agree that if discrepancies exists between sensory results and WBS values, then the WBS value is the less reflective observation when

compared with sensory panel results. Therefore, WBS values should complement sensory panel research and not be used as a sole reflection of palatability (personal communication: Dikeman 1987)

Chemical Measures of Meat Tenderness

Research conducted by investigators from Iowa State University have found that degradation of the Z-line was related to the reduction of fiber tensile strength which is correlated with meat tenderness. Parrish et al. (1973c) found that smaller myofibril fragments originated from more tender meat samples. Over the next several years, this group showed that myofibrils fragmented as postmortem storage length advanced. The extent of myofibrillar fragmentation can be quantified as fragmentation varies with different muscles and different storage temperatures (Olson et al., 1976).

Furthermore, as these investigators began studying proteolyic and ultrastructural relationships involved with meat tenderness, it was discovered that the disappearance of troponin T and increased intensity of a 30 kDa component during advanced postmortem storage was correlated with increased sensory panel tenderness, increased myofibril fragmentation and reduced WBS force (Olson and Parrish, 1977). Subsequent studies have

shown that calcium dependent protease was responsible for this myofibrillar protein degradation.

MacBride and Parrish (1977) reported myofibril fragmentation index is perhaps a most important measurement of tenderness from meat in conventionally aged beef carcasses. Further investigations have confirmed the relationship that myofibril fragmentation index has upon enhancing our understanding of meat tenderness (Olson and Parrish, 1977; Culler et al., 1978).

A recent investigation designed to predict beef tenderness at day 14 postmortem combined mechanical and chemical procedures early postmortem. These investigators incorporated WBS values, myofibrillar fragmentation index procedures and also determined calcium dependent protease inhibitor activity from samples at time of slaughter, again at 24 hours and 24 hours cystatin activity. They found that when combined in a model, these three biochemical markers explained 63 percent of the variation in 14 day WBS values of beef longissimun muscle (Shackelford et al., 1991).

Presently biochemical research designed to evaluate calcium dependent protease inhibitor activity combined with evaluating effects on ultrastructure changes and meat tenderness is being conducted by various groups in the United States and abroad. Further understanding of fundamental mechanisms associated with tenderness will be of benefit to

consumers and all parties involved with the production of livestock and its conversion to meat.

Aspects of Ethanol Metabolism and Cellular Effects

Ethanol can be regarded as an oxidizable substrate as well as a drug. As a food, ethanol is an energy rich source of carbohydrates that is readily absorbed in the gastrointestinal tract. Recent advances in our knowledge of ethanol metabolism has yielded the question of further defining the cellular effects of ethanol ingestion on excitable tissues. Over the last decade, researchers have laid to rest the theory that the cellular effects imparted by ethanol is simply a manifestation of malnutrition. Recent evidence demonstrates that ethanol and its resulting metabolic by-products can directly damage cell components. Research suggests that ethanol has a direct effect on skeletal muscle (Urbano-Marquez et al.,1989). As such, this review will focus on the mechanisms of ethanol metabolism and the cellular changes that occur in skeletal muscle. Throughout this review, ethanol and alcohol are used interchangeably.

Absorption

Alcohol may be classified as a food because it provides energy to the body (McDonald, 1979). In a bomb calorimeter, under optimum termperature and humidity, ethanol yields 7.1 kilocalories per gram (Gillespie and Lucas,

1958). The exact percentage of kilocalories derived from ethanol is debatable because as much as 10% may be lost via urine or exhaled from the lungs (Morgan, 1979) and a small proportion of energy is lost because of heat dissipation (Westerfield and Schulman, 1959). The amount lost in the urine or by the lungs is directly proportional to the concentration of ethanol in the blood (Westerfield and Schulman, 1959).

Alcohol is absorbed throughout the entire length of the gastrointestinal tract (Westerfield and Schulman, 1959). The ethanol molecule is of small size and carries a weak charge. Because of these characteristics, it easily crosses cell membranes and diffuses into the blood (Wilson, 1972). Absorption of ethanol occurs rapidly in the stomach (20%) with a larger percentage being absorbed in the intestines (Pawn, 1972). Dilute solutions (10% - 20%) of ethanol are more readily absorbed than solutions of higher concentration (i.e. > 20%) (Pawn, 1972). Ethanol disappears from the blood linearly with time and is not stored in any body tissues as ethanol (Westerfield and Schulman, 1959). Once absorbed into the body, ethanol is distributed to body tissues proportional to the water content of the cells.

Metabolism

The hepatocyte contains two main pathways for the metabolism of ethanol, each located in a different subcellular compartment: The alcohol

dehydrogenase pathway of the cytosol and the microsomal ethanol-oxidizing system (MEOS) is located in the endoplasmic reticulum.

Alcohol dehyrdrogenase (ADH), an enzyme of the cell cytosol, catalyzes the conversion of ethanol to acetaldehyde. This avenue of metabolism constitutes the major pathway of ethanol catabolism. In ADH-mediated oxidation of ethanol, hydrogen is transferred from the substrate to the cofactor nicotinamide adenine dinucleotide (NAD) resulting in conversion to its reduced form, NADH, with the production of acetaldehyde. Acetaldehyde is eventually oxidized to acetyl CoA. The accumulation of acetyl CoA results in a marked shift in the redox potential of the cytosol, primarily due to the generation of NADH. Acetyl CoA may enter the tricarboxylic acid (TCA) cycle, but is inhibited at the step converting isocitrate to α-ketogluterate. Isocitrate dehydrogenase is inhibited by the high level of NADH causing an accumulation of citrate and acetyl CoA. As a result, the conversion of pyruvate to acetyl CoA is inhibited. Pyruvate metabolism is then shifted to the formation of lactate as measured by changes in the lactate and pyruvate ratio (Domschke et al., 1974; Veech et al., 1972). An elevation of ketone bodies will result with a subsequent occurrence of acetoacetate and B-hydroxy-butyrate when excess acetyl CoA is not allowed to enter the TCA cycle. Glycolytic enzymes are inhibited because of excess citrate and acetyl CoA that act as inhibitory modulators, and because of enzyme competition with ADH for their common cofactor

NAD. Gluconeogenesis is inhibited because of the altered redox state caused by excess NADH.

The microsomal ethanol oxidizing system (MEOS) makes use of the nicotinamide adenine dinucleotide phosphate (NADPH) to active molecular oxygen (Lowry and Griffation, 1972). By utilizing NADPH equivalents, this system actually consumes energy (Pirola, 1978). High levels of NADPH are furnished by such systems as glucose-6-phosphate dehydrogenase, malic enzyme, glutamate dehydrogenase and the hexose monophosphate shunt (Lowry and Griffaton, 1972). The MEOS system may account for as much as 10% of the ethanol metabolism in the body (Anderson, 1978). Accelerated activation of the system occurs at high substrate concentrations.

The reconstitution of the ethanol oxidizing activity with the three microsomal components P-450, NADPH-cytochrome c reductase and lecithin was demonstrated (Ohnishi and Lieber, 1977). The oxidation of ethanol can be mediated by either reductase or cytochrome P-450 (Onishi and Lieber, 1978; Winston and Cederbaum, 1983).

The chronic feeding of ethanol results in increased activity of the MEOS pathway. Ethanol oxidation was enhanced in isolated liver tissue by increasing the in vitro ethanol concentration from 10 to 30 mM (Teschke et al., 1977). Similarly, when a relative constant blood ethanol was maintained through continuous infusion in the baboon, the acceleration of ethanol metabolism with higher blood levels was more pronounced in alcohol fed

than in control animals (Pikkarainen and Leiber, 1980). This data indicates that a non-ADH pathway, most likely MEOS, represents a primary mechanism for the acceleration of ethanol metabolism at high ethanol concentrations.

Biological Effects

Hemodynamics

The hemodynamic changes that occur because of the ingestion of alcohol have been studied by several investigators. Belfrage et al. (1977) studied the effect of ethanol (75 g/day, approximately 15% of total calories) over a five week period. Increases in plasma triglycerides were observed for the first two weeks but returned to normal by week four. There was a significant increases in HDL, a threefold increase in lipoprotein lipase activity with no observed change in LDL. An increase in lipoprotein lipase activity is believed to be a major determinant of lipid removal in the blood. Castelli et al. (1977) found that alcohol consumption was positively correlated with HDL levels in all populations studied. Populations that drank five to six ounces of ethanol per week had 10% higher HDL concentrations when compared to controls.

Body Weight

The energy value of ethanol is calculated at 7.1 kilocalories per gram (Lieber, 1976) compared to 4.0 kilocalories per gram carbohydrate and 9.0 kilocalories per gram fat. Because of its high caloric content, weight gain should result if alcohol is consumed in amounts providing excess calories. Alcohol consumption, along with adequate caloric intake from the diet does not always result in weight gain. A possible explanation for this might be the increased motility and excretion of intestinal contents. Type I peristalic waves (i.e., mixing waves) are often inhibited and Type III waves (i.e., pushing forward) are accelerated when ethanol is consumed (Mezey, 1975). Caloric energy derived from alcohol might also be lost via the lungs, perspiration or through the dissipation of body heat (Gillespie and Lucas, 1958). Shorey et al. (1977) studied the weight patterns of rats consuming an adequate diet with and without alcohol (15% or 20% v/v alcohol), The rats consuming fifteen percent (15%) solutions of alcohol had thirty-three percent (33%) lower weight gains than controls receiving no alcohol. Rats consuming twenty percent (20%) solutions had sixty-four percent (64%) lower weight gains than controls not ingesting alcohol. Chen et al. (1977) demonstrated that ethanol (20% v/v) had no effect on weight patterns of rats fed diets high in carbohydrate (55% starch). Rats fed ethanol with either 10% protein as casein or 5% fat as safflower oil showed significant

decreases in weight gain compared to matched controls containing no ethanol.

Ethanol, a fermentation product of corn silage, is present as 1.5 - 3.5% of the dry matter (Byers et al.,1969; Owens et al., 1969). Byers et al (1979) fed Angus and Hereford X Angus steers a corn silage diet with 3% added ethanol. It was reported that the addition of ethanol improved ADG by 8.5% when adequate selenium was present in the diet. Feed efficiency was not influenced by the addition of ethanol. Adding small quantities of alcohol has been proposed to improve feed utilization. Burroughs et al. (1958) demonstrated that live weight gains were improved by 0.11 pounds per day when cattle received three ounces of alcohol (95% ethanol) daily. The amount of feed per 100 pounds of gain also favored alcohol fed cattle. However, feed cost were not reduced by the alcohol supplementation. Carcass measurements following slaughter were not different across all treatments.

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EFFECTS OF DIETARY ETHANOL ON PERFORMANCE, CARCASS CHARACTERISTICS AND MEAT TENDERNESS OF FEEDLOT STEERS

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ABSTRACT

British crossbred steers (N = 24; initial weight = 472 kg) were limit fed a 91% concentrate diet supplemented with either 0, 2, 4 or 6% of the diet DM as ethanol for 42 d. Ethanol replaced dietary molasses in the diet. Rate and efficiency of gain were slighty, but not significantly, improved by added ethanol but carcass measurements including liver weight and abscess incidence were not affected. Blood alcohol and creatine phosphokinase (CPK, an indicator of muscle damage) concentrations peaked between 14 and 28 days of ethanol consumption but declined thereafter. No structural changes were detected by light or electron micrographs. Ethanol supplementation enhanced the rate of tenderness improvement during aging but failed to significantly improve tenderness at any specific aging time. Rate of tenderness improvement was no greater with 6% than with 4% dietary ethanol.

(Key Words: Feedlot Steers, Ethanol, Tenderness, Aging)

Introduction

Improvement of meat quality is a key issue affecting the beef cattle industry today. As competition for the consumer's food dollar escalates, a priority for those involved in the cattle industry is to use systems to provide consistent and palatable beef (Reagan, 1995). Eating quality is highly dependent on tenderness. This attribute varies more widely than the other palatability characteristics of flavor and juiciness. Advancements toward understanding the relationship between tenderness and the highly dynamic nature of pre-rigor muscle shortening have been appreciable in recent years, however much remains to be done before the results of current research can be confidently applied as a means of increasing consumer satisfaction.

The biochemical events between muscle and meat involve complex metabolic, physical and structural changes. Collectively, these intracellular changes parallel the development of rigor mortis and may elicit much variation in eating quality. Appreciable interest exists in manipulating the contractile events of pre-rigor muscle and accelerating the aging process.

As first described by Locker and Hagyard (1963), pre-rigor beef muscles removed from their skeletal attachments shorten by up to sixty percent (60%) of their initial length when cooled prior to entering the rapid phase of rigor onset at 2°C. This length change associated with cold shortening is accompanied by a several fold toughening (Marsh and Leet, 1966). Interference with the contractual design of muscle in a manner that

would limit pre-rigor shortening would theoretically provide a mechanism to enhance beef quality.

Accelerating the aging process has received considerable interest in recent years. Much of this interest has centered around developing processes which invoke early activation of the aging process by increasing the rate of degradation of the structural integrity of muscle. A serious limitation is the effective regulation of endogenous muscle components. According to Perkoff (1971) and Felix (1982), the sarcolemma can be made permeable to low molecular weight compounds. Additional evidence suggests that related compounds may induce a myopathic condition in the muscle cell (Song and Rubin, 1972). The administration of such compounds may be accomplished by infusing pre-rigor musculature at the time of slaughter (Kauffman, 1987) or by supplementing a high energy ration during the feedlot phase. Although the permeability of the sarcolemma may be enhanced to permit delivery of low molecular weight compounds to individual structural components, no compound that elicits a uniform, rapid and selective alteration has been identified.

Ethanol has a direct effect on muscle (Urbano-Marquez et al, 1989). Either acute or long term consumption of ethanol affects the permeability of the sarcolemma, the integrity of the contractile component and the regulation of contractile activity (Rubin et al., 1976; Puszkin and Rubin, 1976; and Urbano-Marquez et al., 1989). Therefore, by interfering with the contractual

design of muscle, it is conceivable that muscle fibers could remain in a relaxed state, thus limiting muscle shortening and enhancing meat tenderness. Additionally, histological and enzymatic studies suggest that myopathy results from acute or chronic ethanol ingestion (Song and Rubin, 1972; Kahn and Meyer, 1969; Urbano-Marquez et al., 1985). This myopathic condition closely resembles changes that occur to myofibrillar proteins during postmortem aging. This study was designed to determine the effect of dietary alcohol on animal performance, carcass characteristics and meat tenderness.

Materials and Methods

Animals and Diets. Twenty-four crossbred steers nearing market weight (472 kg), were obtained from a single source and constituted the initial pool of animals. Upon arrival at Oklahoma State University, steers were individually weighed, identified and assigned randomly to one of four diets for 42 days. One pen of six steers was fed each diet. Diets consisted of ethanol being supplemented at 0, (controls or basal diet), 2, 4 and 6% of the diet dry matter. Ethanol (95% pure) was substituted 1 to 1 for molasses on a dry matter basis; the sum ethanol and molasses mixture comprised 6.25% of the diet. Diet compositions are shown in Table 1. The ethanol and molasses mixture was prepared at feeding and mixed thoroughly into the feed with every meal. Diets were not isocaloric because ethanol contains

approximately twice the metabolizable energy as molasses. Steers were limit fed (9.88 kg/day) a 91% concentrate ration in two meals daily for 42 days. Dietary treatments were initiated on January 16, 1990. Cattle were fed in feed bunks exposed to the weather.

Animals were allocated based on arrival weight. These were considered to be shrunk weights. Subsequent weights were taken without withdrawal of feed or water. Weight gains and feed efficiency were calculated based on shrunk weights (96% of full weight) to account for gut fill. One steer was removed from the 2% ethanol treatment for reasons not associated with treatment.

Serum Sampling. Serum was collected on the first day of the trial and every fourteen days thereafter to determine blood alcohol and creatine phosphokinase concentrations. Cattle were sampled shortly after their p.m. feeding. Blood alcohol was determined by using a Hewlett Packard 5880 gas chromatograph (Palo Alto, CA). The gas chromatography column was a six foot glass coil with a 4 mm outside diameter. The column was packed with 0.02% Carbowax 1500 on a stationary phase 60/80 mesh Carbopack C. The detection limit for this analytical method is 0.1 mg/dL. Creatine phosphokinase is a mitochondrial enzyme unique to muscle. This enzyme is an index of muscle damage as injured cells leak this enzyme into the blood circulation. Serum creatine phosphokinase concentration was determined spectrophotometrically using a Sigma Diagnostics application (DG147-UV,

St. Louis, MO) for the Roche Cobas Mira chemistry system (Branchburg, NJ). Absorbance was measured at 340 nm.

Carcass Data and Muscle Sampling. At the conclusion of the feeding trial, cattle were slaughtered at the Oklahoma State University meat laboratory. Carcasses were not subjected to electrical stimulation at time of slaughter. Livers were weighed and examined for the presence and severity of liver abscesses. After carcasses were allowed to chill for approximately 24 hours, complete yield and quality grade data (USDA, 1989) were recorded.

Following the collection of carcass data, longissimus, semimembranosus, semitendinosus, biceps femoris and gluteus medius samples were fabricated and collected from the left side of each carcass. To prepare longissimus samples, individual strip loins (IMPS 180) were faced by removing the uneven 12th rib portion. A 1.3 cm thick steak was removed from the anterior end of the strip loin, denuded of exterior fat and connective tissue, vacuum packaged and stored at -30°C until proximate composition was determined. Immediately anterior to the steak used for proximate analysis, five steaks 2.54 cm thick were cut and vacuum packaged to assess cooking properties and tenderness. Longissimus steaks were assigned to serial aging treatments; the first of these steaks from the posterior end was cooler aged for 3d, the second for 7d, the third for 14d, the fourth for 28d, and the fifth for 42d.

Semimembranosus and semitendinosus samples were collected after fabrication of beef rounds (IMPS 158). Muscles were faced by removing the uneven portion. A 2.54 cm steak was removed from the anterior end of each muscle and vacuum packaged to evaluate cooking properties and tenderness characteristics. These steaks were cooler aged for 7 days.

Top sirloin butts (IMPS 184) were fabricated to yield biceps femoris and gluteus medius samples. A 2.54 cm steak was removed from the posterior end of each muscle and vacuum packaged to evaluate cooking properties and tenderness characteristics. These steaks were cooler aged for 7 days.

Cooler temperature was maintained at $+1^{\circ}C \pm 2^{\circ}C$ throughout aging periods. Aged steaks were stored at $-30^{\circ}C$ until cooking properties and tenderness characteristics were determined.

Proximate Analysis. Duplicate longissimus samples were analyzed according to procedures outlined by AOAC (1984). Samples were immersed in liquid nitrogen and subsequently powdered in a Waring® model 34BL22 commercial blender (New Hartford, CT). A 3 g powdered sample was dried at $104^{\circ}C$ for 24 hours and cooled in a desiccator for 1 hour. Samples were weighed to determine their moisture content. Following moisture determination, samples were placed in a soxhlet and extracted for 24 hours with petroleum ether. Samples were dried at $104^{\circ}C$ for 12 hours, cooled in a desiccator and weighed to determine lipid content. Crude protein was

determined using the Kjeldahl method. A .5 g powdered sample was placed in digestion tubes with two Kjel® tabs (3.5 g potassium sulfate + 0.0035 g Selenium) and digested for 2 hours at 420°C. Samples were removed, extended with 75 ml of deionized water and analyzed for protein using a KJELTEC® 1030 Auto Analyzer (Hoganas, Sweden).

Steak Cookery and Shear Force. Cooking properties and shear force determinations were performed as described in AMSA (1978). Aged steaks were allowed to thaw at 3°C for 17 hours and broiled on Faberware® (Bronx, NY) open-hearth broilers to a final internal temperature of 70°C. Constantan coated copper thermocouples were placed in the geometric center of each steak. Internal temperature was monitored using a OMEGA® OM-302 temperature logger (Stamford, CT). Cooking time to a medium degree of doneness (minutes/100 g raw steak) and cooking loss (percentage weight loss) were determined for each steak. Steaks were allowed to cool to 25°C after which 6-8 cores measuring 1.27 cm in diameter were removed parallel to the longitudinal axis of the muscle fibers. Individual cores were objectively measured for tenderness using the Warner-Bratzler shear apparatus attached to a Instron® Universal Testing Machine Model 4502 (Canton, MA).

Data Analysis. A completely randomized design was used in which animals were stratified by initial weight. Animal performance, carcass and palatability characteristics were analyzed using individual animals as

experimental units using the general linear model of SAS (SAS, 1995). Animal performance and carcass characteristic effects of ethanol treatments were determined. Ethanol treatment, muscle and age were included as classes in the statistical model for cooking properties and shear force. No two or three interactions ($P>.05$) were detected. Least square means are reported to account for the unequal number of steers per treatments. Additionally, orthogonal comparisons were utilized to examine linear, quadratic and cubic effects. Significance is reported at the .05 level unless noted otherwise.

Results and Discussion

A preliminary concern was the palatability and evaporative properties of ethanol. Prior to starting the trial, test batches of feed were prepared and weighed every five minutes for the first hour and then every hour for twelve hours. Weight loss by evaporation was not different across dietary treatments indicating that evaporative loss of ethanol was minor. When the diets were first fed, a one to two day eating adaptation period was noticed among the ethanol supplemented groups. Immediately after ethanol was added, the feed became cool to the touch and it had a strong, sweet smell. During the study, the time required for feed consumption was not numerically different among diets. Presumably, ethanol was not detrimental to ration palatability because bunks were slick clean of feed after each

feeding. Feed was completely consumed within 20 to 40 minutes. Steers receiving ethanol seemed more docile and easier to handle when processing.

Steer Performance. Effects of ethanol supplementation on cattle performance are reported in Table 2. Initial weight and final weight were not significantly different between ethanol treatments. Average daily gain and feed efficiency tended to be improved when ethanol was supplemented to limit fed steers. Average daily gain (live weight basis) was 36% greater ($P<0.09$) when ethanol comprised 6% of the diet when compared to controls. Feed conversion (feed/gain) was improved from 8.22 (controls) to 7.25 when ethanol was supplemented at the 6% level. Burroughs et al., (1958) observed that 64g of ethanol daily, about .6% of diet DM, improved live weight gains of cattle. Alcohol supplemented cattle required 3% less feed per pound of liveweight gain (Burroughs et al., 1958). Ethanol, a natural fermentation product of corn silage, is present as 1.5-3.5% of the dry matter (Byers et al., 1969; Owens et al., 1969). Byers et al. (1979) fed Angus and Hereford x Angus steers a corn silage diet with 3% added ethanol. Average daily gain was 8.5% greater when adequate selenium was present in the diet but feed efficiency was not influenced by added ethanol. Consumption of alcohol together with adequate caloric intake does not always increase weight gain. Ethanol may be increase motility and excretion of intestinal contents. Type I peristalic waves (i.e., mixing waves) often are inhibited and Type III waves (i.e., pushing forward) are accelerated when

ethanol is consumed (Mezey, 1975). Interestingly, Chen et al. (1977) demonstrated that ethanol supplementation (20% v/v) had no effect on weight gain of rats fed carbohydrate diets.

Blood Analysis. Effects of ethanol on blood alcohol and creatine phosphokinase levels are shown in Table 3. Cattle were bled shortly after their p.m. feeding. Initial blood alcohol levels were below the detection limits of the analytical method. After 14 days on trial, steers receiving 4% ethanol supplementation had higher ($P<.05$) blood alcohol levels than cattle fed other ethanol levels. This trend continued throughout the trial. Blood alcohol levels peaked between day 14 and day 28 and declined gradually thereafter. This may represent metabolic adaptation by the animal. Higher intakes may be required over time to maintain blood levels. Similar time/dose conclusions with ethanol and related drugs are well documented in humans, but such a response to ethanol has not been reported in cattle.

Histological and enzymatic studies suggest that a myopathic condition results from acute and chronic ethanol ingestion (Song and Rubin, 1972; Kahn and Meyer, 1969; Urbano-Marquez et al., 1985). This myopathic condition closely resembles changes that occur to myofibrillar proteins during postmortem aging. Serum creatine phosphokinase was elevated linearly ($P<.02$) on day 14 of the study. Thereafter, values tended to be higher with 6% dietary ethanol. This is consistent with muscle damage. However, electron microscopy detected no atrophic effect on tissue morphology in this

study. Experiments with non-ruminants have reported serum creatine phosphokinase concentrations to increase after either chronic or acute ethanol ingestion (Rubin, 1979; Song and Rubin, 1972; Diamond, 1989). Interestingly, creatine phosphokinase levels appeared to be markedly elevated at the beginning of the trial and highest at slaughter. Higher values at these times may represent muscular damage associated with livestock handling and transportation.

Carcass traits. Carcass characteristics were not different ($P>.05$) across dietary treatments (Table 4). These results are in agreement with Burroughs et al. (1958). However, carcass weights for steers receiving 6% ethanol tended to be the greatest. Steers supplemented with 2 and 6% ethanol were slightly leaner and more muscular as indicated by slightly larger ribeye area measurements and slightly less fat thickness over the rib. Ethanol addition to various preparations has inhibited protein synthesis in vitro (Joly et al., 1977). In vivo, effects of chronic or acute ethanol ingestion on protein synthesis have not been consistent. (Lieber, 1984). USDA yield grade data from our study revealed that steers supplemented with 2 and 6% ethanol tended to have more carcasses with yield grades of 2.9 or better (cubic effect, $P=.20$). However, the percentage of carcasses attaining the U.S. Choice grade tended to decrease with ethanol supplementation. Overall maturity scores were slightly more advanced for cattle fed ethanol.

Because ethanol is metabolized in the liver, consumption directly affects liver function. Addition of ethanol to the diet of these steers did not alter weight or liver abscess incidence.

Composition, Cooking Properties and Shear Force. Dietary treatments did not significantly alter the proximate composition of longissimus samples although percent protein tended ($P<.07$) to increase linearly as the level of ethanol increased (Table 5). Depending on the fibrous protein of interest, ethanol has been reported to induce redox changes that may either impair or enhance the rate of protein synthesis (Lieber, 1984). Muscle samples from cattle fed two and four percent ethanol had slightly less lipid content. In short term studies, administration of ethanol has depressed free fatty acids in plasma of nonruminants (Lieber et al., 1962), decreased fatty acid turnover (Jones et al., 1965) and reduced circulating glycerol (Feinman and Lieber, 1967). Acetate, the end product of ethanol metabolism by the liver, is released into the blood. Since acute doses both stimulate fatty acid mobilization via catecholamine release and depress it via acetate production, the net effect on muscle composition may depend upon the specific experimental conditions (Lieber, 1984).

Cooking shrink and cooking time results for longissimus samples are presented in Table 6. Laakonen et al. (1970) reported that degree of doneness in meat is extremely critical when measuring tenderness and cooking yield. With the exception of longissimus steaks aged 14 d, no

treatment differences ($P>.05$) were detected in cooking shrink and cooking time. For longissimus steaks aged 14 d, cooking losses were lower for steaks from 2 and 4% ethanol treatments when compared to controls. Cooking time to reach a 70°C endpoint temperature decreased ($P<.02$) linearly for steaks aged 7 d with increasing dietary ethanol. Quadratic responses were evident for cooking shrink when longissimus steaks were aged 3 ($P<.02$), 14 ($P<.007$) and 42 d ($P<.09$). These results suggest that with moderate (2% and 4%) intakes of ethanol, longissimus steaks lost less juices during cooking.

Tenderness of longissimus steaks was measured by determining Warner Bratzler shear force values at the five aging times. Results are shown in Table 7. Aging of meat is a commercially important process to increase tenderness. Industry storage criteria typically is 0-3°C for 7-14 days, however, the logistics of inventory and storage facilities often shorten this time. Morgan et al. (1991) found that the average post-fabrication time, defined as the time for primals and sub-primals to arrive to retail outlets, for all cuts was approximately 17 d.

Warner Bratzler shear force and percent tough, tender and very tender steaks in our study was not altered ($P>.05$) by ethanol diets. Aging of steaks consistently decreased Warner Bratzler shear values across all dietary treatments. Aging effects became significant after 28 d for control animals, 14 d for steers fed 2% ethanol and 7 d for steers receiving 4 or 6% ethanol.

By day 7, tenderness was improved by 6.8, 8.2, 25.7 and 17.3% for control, 2, 4 and 6% dietary ethanol treatments, respectively. Tenderness improvements from d 3 to d 14 were 27.5, 36.1, 57.7 and 48.5% for these four dietary treatments. These data suggest that the rate of postmortem tenderization is accelerated by increased intake of ethanol although the rate of improvement was similar for the 4 and 6% dietary treatments.

Several cellular effects of ethanol may explain these results. Urbano-Marquez et al. (1989) eloquently showed that ethanol has a direct effect on skeletal muscle. The acute or long term administration of ethanol affects contractile component and regulation of contractile activity of the myofibril. Additionally, the ATPase activity and actomyosin complex is altered by low concentrations of ethanol such that the muscle can not develop normal tension (Puszkin and Rubin, 1976), possibility due to the inability of the ATPase receptor sites to adequately bind calcium. Interference with the contractual design of skeletal muscle may limit rigor shortening and thereby improve tenderness.

One factor of concern in this study was variation among core samples within steaks. Table 8 displays the effects of ethanol on shear force standard deviations among core samples within each longissimus steak. Core to core variation was not altered ($P>.05$) by dietary treatment.

Table 9 presents the effects of ethanol on cooking properties and shear force values for semimembranosus muscles aged 7 d. Cooking shrink

was not altered (P>.05) but cooking time was reduced linearly (P<.01) and quadratically by dietary ethanol. Similar to longissimus steaks aged 7 d, the tenderness of semimembranosus steaks was not altered by dietary treatment. More than eighty percent of all semimembranosus steaks were considered to be tough (> 4.54 kg shear force).

Semitendinosus cooking shrink, cooking times and shear force were not altered (P>.05) by feeding ethanol (Table 10). As noted for semimembranosus steaks in Table 9, over 80 percent of semitendinosus steaks were classified as tough when aged 7 d. However, in this case, ethanol slightly, but not significantly, increased the percentage of semitendinosus steaks classified as tender.

Cooking properties and shear values for Biceps femoris and Gluteus medius steaks aged 7 d are shown in Table 11. Ethanol intake did not significantly alter (P>.05) any of the dependent variables. Interestingly, in this case, cattle consuming the basal diet actually had the highest numeric percentage of tender steaks. Numerically, Bicep femoris steaks from cattle consuming the 2% dietary treatment had the lowest average shear force value whereas for Gluteus medius steaks, shear force values were greatest for the 2% dietary treatment. Comparisons of tables 7, 9, 10 and 11 show no differential effect on tenderness of steaks aged 7 d from different muscle locations.

Presented in Table 12 are the standard deviations within steaks based on core to core differences in shear force. As noted with longissimus steaks, core to core variation was not altered by dietary treatment at each muscle location.

Implications

Supplementing beef cattle finishing rations with ethanol may improve the rate at which postmortem tenderness improves even though it failed to increase tenderness at any specific time. However, enhancing the rate of tenderization decreases postmortem storage time. Further studies are needed on the effects of ethanol on the consistency and tenderness of beef.

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Table 1. Composition of diets (dry matter basis)

	Ethanol (%)			
Ingredient (%)	0	2	4	6
Corn, rolled	78.65	78.31	77.98	77.65
Alfalfa hay, pelleted	4.06	4.05	4.03	4.01
Cottonseed hulls	5.06	5.04	5.02	5.00
Pelleted supplement[a]	7.19	7.16	7.13	7.10
Molasses, cane	5.03	3.41	1.80	0.20
Ethanol[b]	0	2.03	4.04	6.04
Calculated composition				
NEm, Mcal/kg[c]	2.07	2.11	2.15	2.19
NEg, Mcal/kg[c]	1.32	1.35	1.38	1.42
Crude protein, %	12.08	11.93	11.77	11.62
K, %	0.73	0.66	0.60	0.53
Ca, %	0.52	0.50	0.48	0.47
P, %	0.33	0.33	0.32	0.32

[a] Supplement composition (%): Cottonseed meal, 75.92; calcium carbonate, 12.05; urea, 5.69; salt, 4.23; dicalcium phosphate, 1.18; Rumensin 60®, 0.27; vitamin E, 0.21; Tylan®, 0.17; vitamin A, 0.15; trace mineral pre-mix, 0.13.

[b] Ethanol mixed into ration was 90% pure (180 proof).

[c] Ethanol provides 5.30 kcal NEm/kg and 3.94 kcal NEg/kg.

Table 2. Effects of ethanol on performance of feedlot steers[a]

	Ethanol (%)					Observed significance (P <)		
	0	2	4	6	SEM	Linear	Quadratic	Cubic
Number of steers[b]	6	5	6	6				
Initial weight, kg	467.7	476.3	475.1	475.9	11.55	0.64	0.73	0.82
Final weight, kg	516.6	533.5	534.9	543.7	17.89	0.29	0.82	0.78
Daily gain, kg	1.33	1.64	1.73	1.81	0.21	0.09	0.59	0.81
Feed intake, kg	9.98	9.98	9.98	9.98				
Feed/gain	8.22	6.53	5.86	7.25	1.44	0.57	0.29	0.87
ME, Mcal/kg DM	2.70	2.99	3.04	3.12				
NEm, Mcal/kg DM	1.78	2.02	2.07	2.13				
NEg, Mcal/kg DM	1.25	1.36	1.40	1.46				

[a] Least square means; SEM n = 5.48.

[b] One steer was removed from the 2% ethanol treatment for reasons not associated with dietary treatments.

Table 3. Effects of ethanol on blood alcohol (BA) and creatine phosphokinase (CPK) levels of feedlot steers[a]

	Ethanol (%)					Observed significance (P <)		
	0	2	4	6	SEM	Linear	Quadratic	Cubic
Number of steers[b]	6	5	6	6				
BA, mg/dL blood								
Day 1	0	0	0	0				
Day 14	0[d]	0[d]	19.13[c]	1.03[d]	3.54	0.16	0.02	0.002
Day 28	0[d]	0.40[d]	13.50[c]	0.93[d]	3.80	0.34	0.10	0.03
Day 42	0[d]	0[d]	3.30[c]	0.20[d]	0.45	0.06	0.002	0.0001
CPK, IU/L blood								
Day 1	217.4	192.4	159.2	192.5	41.07	0.57	0.50	0.70
Day 14	102.5	120.3	144.7	162.2	18.94	0.02	0.99	0.89
Day 28	104.2	92.0	101.3	152.2	20.83	0.10	0.14	0.83
Day 42	104.0	97.4	88.2	118.2	14.44	0.60	0.21	0.52
Slaughter	729.7	445.4	449.2	697.4	132.39	0.88	0.07	0.94

[a] Least square means; SEM n = 5.48.

[b] One steer was removed from the 2% ethanol treatment for reasons not associated with dietary treatments.

[c,d] Means in the same row with a common superscript letter are not different (P > .05).

Table 4. Effects of ethanol on carcass characteristics of feedlot steers[a]

	Ethanol (%)					Observed significance (P <)		
	0	2	4	6	SEM	Linear	Quadratic	Cubic
Number of steers[b]	6	5	6	6				
Carcass weight, kg	334.0	344.6	335.8	351.0	10.56	0.36	0.83	0.37
Dressing percentage	64.7	64.6	62.7	64.9	1.09	0.77	0.30	0.25
Fat thickness, cm	1.63	1.24	1.76	1.34	0.20	0.70	0.93	0.05
Ribeye area, cm^2	86.3	89.7	83.7	92.8	4.96	0.52	0.57	0.28
KPH fat, %	2.3	3.0	2.4	2.5	0.27	0.97	0.33	0.13
USDA yield grade	3.1	2.8	3.4	2.6	0.38	0.70	0.62	0.20
Percent yield grade 4	16.7	0	33.3	16.7	16.98	0.65	1.00	0.20
Maturity score[c]								
Skeletal	158	168	158	188	15.66	0.25	0.51	0.41
Lean	150	162	168	167	16.08	0.42	0.67	0.97
Overall	154	165	163	178	15.31	0.31	0.91	0.68
Marbling score[d]	482	454	468	423	48.58	0.45	0.86	0.64
Percent choice	66.7	80.0	66.7	50.0	21.07	0.51	0.49	0.81
Condemned liver, %	0	0	0	0				
Liver weight, kg	7.2	7.0	6.8	7.2	0.65	0.95	0.36	0.65

[a] Least square means; SEM n = 5.48.

[b] One steer was removed from the 2% ethanol treatment for reasons not associated with dietary treatments.

[c] Maturity score: 100-199 = A, approximately 9-30 months of age.

[d] Marbling score: 400 = Small 00, minimum for USDA Choice.

Table 5. Effects of ethanol on Longissimus proximate composition[a]

	Ethanol (%)					Observed significance (P <)		
	0	2	4	6	SEM	Linear	Quadratic	Cubic
Number of steers[b]	6	5	6	6				
Protein, %	20.7	21.2	21.3	21.5	0.29	0.07	0.53	0.64
Moisture, %	73.3	74.0	73.3	73.9	0.66	0.67	0.93	0.36
Lipid, %	4.2	3.6	4.2	3.5	0.74	0.64	0.91	0.47

[a] Least square means; SEM n=5.48.

[b] One steer was removed from the 2% ethanol treatment for reasons not associated with dietary treatments.

Table 6. Effects of ethanol on Longissimus cooking properties[a]

	Ethanol (%)					Observed significance (P <)		
	0	2	4	6	SEM	Linear	Quadratic	Cubic
Number of steers[b]	6	5	6	6				
Aged 3 days								
Cooking shrink, %	31.46	28.13	29.05	31.43	1.18	0.87	0.02	0.60
Cooking time[c]	9.37	8.63	8.71	8.60	0.50	0.31	0.53	0.66
Aged 7 days								
Cooking shrink, %	28.11	28.91	29.05	28.27	0.91	0.88	0.40	0.95
Cooking time	10.07	9.08	8.97	8.00	0.55	0.02	0.99	0.49
Aged 14 days								
Cooking shrink, %	29.99[d]	27.25[e]	27.22[e]	28.88[de]	0.74	0.30	0.007	0.76
Cooking time	9.00	8.15	8.88	8.74	0.44	0.98	0.43	0.23
Aged 28 days								
Cooking shrink, %	26.22	27.78	26.36	27.83	1.68	0.64	0.98	0.44
Cooking time	7.82	8.35	8.01	7.58	0.59	0.68	0.41	0.77
Aged 42 days								
Cooking shrink, %	30.25	27.87	28.45	31.07	1.45	0.63	0.09	0.89
Cooking time	9.01	7.23	8.47	7.87	0.73	0.49	0.42	0.15

[a] Least square means. SEM n = 5.48.

[b] One steer was removed from the 2% ethanol treatment for reasons not associated with dietary treatments.

[c] Cooking time expressed as minutes to 70°C/100 g raw steak.

[d,e] Means in the same row with a common superscript letter are not different (P > .05).

Table 7. Effects of ethanol on Longissimus shear force values[a]

	Ethanol (%)					Observed significance (P <)		
	0	2	4	6	SEM	Linear	Quadratic	Cubic
Number of steers[b]	6	5	6	6				
Aged 3 days								
Shear force, kg	5.47	5.28	5.33	5.08	0.44	0.55	0.94	0.79
Tough[f], %	83.33	80.00	66.67	83.33		0.87	0.60	0.64
Tender[g], %	16.67	20.00	16.67	16.67		0.97	0.92	0.90
Very tender[h], %	0	0	16.67	0		0.67	0.35	0.23
Aged 7 days								
Shear force, kg	5.12[c]	4.88[c]	4.24[d]	4.33[d]	0.47	0.15	0.72	0.59
Tough, %	66.67	60.00	50.00	50.00		0.55	0.88	0.90
Tender, %	0	40.00	0	33.33		0.38	0.83	0.04
Very tender, %	33.33	0	50.00	16.67		1.0	1.0	0.06
Aged 14 days								
Shear force, kg	4.29[cd]	3.88[d]	3.49[de]	3.42[e]	0.41	0.10	0.67	0.87
Tough, %	16.67	20.00	0	0		0.21	0.89	0.45
Tender, %	33.33	0	16.67	16.67		0.65	0.33	0.39
Very tender, %	50.00	80.00	83.33	83.33		0.23	0.44	0.79
Aged 28 days								
Shear force, kg	3.42[c]	3.29[c]	2.73[d]	2.78[d]	0.31	0.08	0.78	0.46
Tough, %	16.67	0	0	0		0.21	0.35	0.68
Tender, %	0	20	0	16.67		0.58	0.89	0.19
Very tender, %	83.33	80.00	100.00	83.33		0.77	0.66	0.39

Aged 42 days								
Shear force, kg	3.37[c]	2.97[c]	2.84[d]	2.84[d]	0.25	0.13	0.43	0.92
Tough, %	16.67	0	0	0		0.21	0.35	0.68
Tender, %	0	0	0	0				
Very tender, %	83.33	100.00	100.00	100.00		0.21	0.35	0.68

[a] Least square means. SEM n = 5.48.

[b] One steer was removed from the 2% ethanol treatment for reasons not associated with dietary treatments.

[c,d,e] Means in the same column with a common superscript letter are not different ($P > .05$).

[f] Percentage of steaks with shear force values of 4.54 kg or higher.

[g] Percentage of steaks with shear force values between 3.86 and 4.53 kg.

[h] Percentage of steaks with shear force values less than 3.86 kg.

Table 8. Effects of ethanol on Longissimus shear force standard deviations[a]

	Ethanol (%)					Observed significance (P <)		
	0	2	4	6	SEM	Linear	Quadratic	Cubic
Number of steers[b]	6	5	6	6				
Standard deviation								
Aged 3 days	2.16	2.10	2.16	2.11	0.12	0.87	0.97	0.68
Aged 7 days	1.93	2.22	2.11	2.06	0.07	0.35	0.03	0.17
Aged 14 days	2.16	2.28	2.26	2.11	0.10	0.72	0.22	0.98
Aged 28 days	2.21	2.22	2.26	2.02	0.11	0.22	0.22	0.50
Aged 42 days	2.21	2.22	2.35	2.30	0.08	0.25	0.72	0.41

[a] Least square means. SEM n = 5.48.

[b] One steer was removed from the 2% ethanol treatment for reasons not associated with dietary treatments.

Table 9. Effects of ethanol on Semimembranosus cooking properties and shear force values aged 7 days[a]

	Ethanol (%)					Observed significance (P <)		
	0	2	4	6	SEM	Linear	Quadratic	Cubic
Number of steers[b]	6	5	6	6				
Cooking properties								
Cooking shrink, %	38.59	38.25	38.84	37.87	1.28	0.79	0.81	0.67
Cooking time[c]	17.91[d]	15.15[e]	13.62[e]	14.72[e]	0.83	0.008	0.03	0.71
Shear force, kg	5.36	5.15	5.67	5.34	0.19	0.60	0.75	0.07
Tough[f], %	83.33	80.00	100.00	100.00		0.21	0.89	0.45
Tender[g], %	0	20	0	0		0.60	0.26	0.14
Very tender[h], %	16.67	0	0	0		0.21	0.35	0.68

[a] Least square means. SEM n = 5.48.

[b] One steer was removed from the 2% ethanol treatment for reasons not associated with dietary treatments.

[c] Cooking time expressed as minutes to 70°C/100 g raw steak.

[d,e] Means in the same row with a common superscript letter are not different (P > .05).

[f] Percentage of steaks with shear force values of 4.54 kg or higher.

[g] Percentage of steaks with shear force values between 3.86 and 4.53 kg.

[h] Percentage of steaks with shear force values less than 3.86 kg.

Table 10. Effects of ethanol on Semitendinosus cooking properties and shear force values aged 7 days[a]

	Ethanol (%)					Observed significance (P <)		
	0	2	4	6	SEM	Linear	Quadratic	Cubic
Number of steers[b]	6	5	6	6				
Cooking properties								
Cooking shrink, %	37.21	35.53	36.57	35.61	1.62	0.61	0.83	0.52
Cooking time[c]	16.48	15.42	15.03	13.29	1.04	0.05	0.75	0.67
Shear force, kg	5.63	5.24	5.40	5.43	0.41	0.82	0.61	0.71
Tough[d], %	83.33	80.00	83.33	83.33		0.97	0.92	0.90
Tender[e], %	0	20.00	16.67	16.67		0.49	0.52	0.70
Very tender[f], %	16.67	0	0	0		0.21	0.35	0.68

[a] Least square means. SEM n = 5.48.

[b] One steer was removed from the 2% ethanol treatment for reasons not associated with dietary treatments.

[c] Cooking time expressed as minutes to 70°C/100 g raw steak.

[d] Percentage of steaks with shear force values of 4.54 kg or higher.

[e] Percentage of steaks with shear force values between 3.86 and 4.53 kg.

[f] Percentage of steaks with shear force values less than 3.86 kg.

Table 11. Effects of ethanol on Top sirloin butt cooking properties and shear force values aged 7 days[a]

	Ethanol (%)					Observed significance (P <)		
	0	2	4	6	SEM	Linear	Quadratic	Cubic
Number of steers[b]	6	5	6	6				
Cooking properties								
Cooking shrink, %	28.22	28.69	31.51	29.26	1.97	0.53	0.52	0.43
Cooking time[c]	3.76	4.10	3.90	3.74	0.38	0.02	0.32	0.08
Biceps femoris								
Shear force, kg	4.11	3.71	4.19	4.13	0.27	0.67	0.55	0.27
Tough[d], %	0	0	16.67	16.67		0.23	1.0	0.56
Tender[e], %	66.67	40.00	33.33	33.33		0.28	0.55	0.90
Very tender[f], %	33.33	60.00	50.00	50.00		0.69	0.56	0.66
Gluteus medius								
Shear force, kg	4.88	5.43	4.91	5.24	0.36	0.73	0.77	0.27
Tough, %	66.67	100.00	66.67	66.67		0.70	0.40	0.27
Tender, %	16.67	0	0	33.33		0.43	0.09	0.80
Very tender, %	16.67	0	33.33	0		0.79	0.56	0.09

a Least square means. SEM n = 5.48.

b One steer was removed from the 2% ethanol treatment for reasons not associated with dietary treatments.

c Cooking time expressed as minutes to 70°C/100 g raw steak.

d Percentage of steaks with shear force values of 4.54 kg or higher.

e Percentage of steaks with shear force values between 3.86 and 4.53 kg.

f Percentage of steaks with shear force values less than 3.86 kg.

Table 12. Effects of ethanol on Semimembranosus, Semitendinosus, Biceps femoris and Gluteus medius shear force standard deviations aged 7 days[a]

	Ethanol (%)					Observed significance (P <)		
	0	2	4	6	SEM	Linear	Quadratic	Cubic
Number of steers[b]	6	5	6	6				
Standard deviation								
Semimembranosus	2.28	2.33	1.60	2.45	0.10	0.46	0.28	0.15
Semitendinosus	2.33	2.28	2.40	2.40	0.07	0.32	0.69	0.35
Biceps femoris	1.93	1.87	1.93	1.82	0.10	0.56	0.81	0.54
Gluteus medius	2.33	2.10	2.28	2.21	0.11	0.69	0.49	0.23

[a] Least square means. SEM n = 5.48.

[b] One steer was removed from the 2% ethanol treatment for reasons not associated with dietary treatments.

EFFECTS OF FEEDING ETHANOL ON PERFORMANCE OF FEEDLOT STEERS, CARCASS CHARACTERISTICS AND MEAT TENDERNESS

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ABSTRACT

Steers (n = 126; 286 kg) were blocked by initial weight and fed a high concentrate diet supplemented with 4% of the diet DM as ethanol for either 0, 17, 24, 38, 66 or 122 days prior to slaughter. Performance and carcass traits were not significantly altered by feeding ethanol. Creatine phosphokinase concentrations also were not altered by ethanol. Cattle fed ethanol for 24 d exhibited the largest improvement in tenderness of the Longissimus muscle. Ethanol supplementation may improve the rate of postmortem aging even though steaks were as tender initially from control steers as from steers fed ethanol.

(Key Words: Feedlot steers, Ethanol, Tenderness, Aging)

Introduction

Inconsistent eating quality, lack of tenderness in particular, limits the consumer acceptance of beef (Reagan, 1995; Morgan et al., 1991). The biochemical changes that occur in conversion of muscle to meat are complex and involve metabolic, physical and structural changes and may be responsible for much of the variation in eating quality. Altering muscle contraction and accelerating the aging process may enhance tenderness. Locker and Hagyard (1963) reported that pre-rigor beef muscles removed from their skeletal attachments shortened by up to sixty percent (60%) of

the initial length when cooled at 2°C prior to entering the rapid phase of rigor onset. Such "cold shortening" is accompanied by toughening by several orders of magnitude (Marsh and Leet, 1966). Interference with this contraction should limit pre-rigor shortening and improve tenderness. Aging also improves tenderness. The aging process can be accelerated by destruction of the structural integrity of muscle. Perkoff (1971) and Felix (1982) indicated that the sarcolemma can be made permeable to low molecular weight compounds which in turn may induce a myopathic condition in the muscle cell (Song and Rubin, 1972). Such compounds can be administered at the time of slaughter through infusion (Kauffman, 1987) or by supplementing a high energy ration during the feedlot phase. Because the sarcolemma limits delivery of low molecular weight compounds to individual structural components, no compound has yet been identified that elicits a uniform, rapid and selective myopathy.

Ethanol has a direct acute and long-term effects on muscle (Urbano-Marquez et al, 1989). By altering the permeability of the sarcolemma, ethanol alters the integrity of the contractile component and regulation of contractile activity (Rubin et al., 1976; Puszkin and Rubin, 1976; and Urbano-Marquez et al., 1989). By maintaining muscle fibers in a non-contractile condition, muscle shortening will be reduced and meat tenderness enhanced. Histological and enzymatic studies both indicate acute and chronic ethanol ingestion cause myopathy (Song and Rubin, 1972; Kahn and Meyer, 1969; Urbano-Marquez et al., 1985) that closely resembles changes that occur to myofibrillar proteins during postmortem aging. This study was devised to examine the effect of ethanol on feedlot performance, carcass characteristics, and, in particular, meat tenderness.

Materials and Methods

Animals and Diets. One hundred twenty-six yearling crossbred steers (285 kg initially) were obtained; approximately 30% of these steers based on visual appraisal carried Brahman genetics. Upon arrival at Oklahoma State University, each steer was weighed, identified, processed and placed in one of three weight blocks of 42 steers each. Initial processing consisted of IBR-PI3-Lepto, 4-way clostridial vaccination, deworming with Ivermectin and implanting with Compudose-200®. Within each weight block, one pen of cattle was assigned each of six treatments for a total of 3 pens (21 steers) per treatment. Treatments consisted of ethanol being fed as 4% of the diet DM for either 0, (controls) 17, 24, 38, 66 or 122 days prior to slaughter.

Steers were given ad libitum access to feed; ethanol and fresh feed was provided twice daily. Steers were fed in a confinement facility. The proportional amount of ethanol was mixed thoroughly into the feed with every meal. Cottonseed hulls and chopped alfalfa, used as roughage sources, were removed in a stepwise fashion to adapt steers to their final diet. Steers were receiving their final ration by day 28 of the study. Dietary treatments were initiated on November 8, 1990. Net energy values were calculated for the control and 4% ethanol diets using the yearling steer equations as reported by Hays et al. (1986). Final diet compositions are shown in Table 1.

Initial allocation weights were obtained directly off the truck and were considered to be shrunk weights. Subsequent weights were taken without withdrawal of feed or water. Weight gains and feed efficiency were based on shrunk weights (calculated as 96% of full weight to account for gut fill).

Serum Sampling. Blood was collected on day 1, 56 and 120 of the trial to determine serum alcohol and creatine phosphokinase concentrations.

Blood alcohol was determined by using a Hewlett Packard 5880 gas chromatograph (Palo Alto, CA). The gas chromatographic column was a six foot glass coil with a 4 mm outside diameter packed with 0.02% Carbowax 1500 on a stationary phase of 60/80 mesh Carbopack C. The detection limit for this analytical method is 0.1 mg/dL. Creatine phosphokinase, a mitochondrial enzyme unique to muscle, serves as an index of muscle damage; this enzyme leaks from injured cells into the blood circulation. Serum creatine phosphokinase concentration was determined spectrophotometrically using a Sigma Diagnostics application (DG147-UV, Sigma Chemical Co., St. Louis, MO) using the Roche Cobas Mira chemistry system (Branchburg, NJ). Absorbance was measured at 340 nm.

Carcass Data and Muscle Sampling. At the conclusion of the feeding trial, cattle were transported to Dodge City, KS for slaughter. Carcasses were subjected to electrical stimulation at time of slaughter. Livers were examined for the presence and severity of liver abscesses. After carcasses were chilled for approximately 48 hours, yield and quality grade data (USDA, 1989) were obtained.

Following the collection of carcass data, longissimus and semimembranosus muscles were obtained from the left side of each carcass. A 20 cm thick section of the longissimus muscle, corresponding to the 9th through 12th ribs, was collected and vacuum packaged. Beef inside rounds (IMPS 168) were removed and vacuum packaged to yield semimembranosus samples. Both muscle sections were transported to Oklahoma State University meat laboratory for fabrication.

To prepare longissimus samples, rib sections were faced by removing the uneven 12th rib portion. A 1.3 cm thick steak was removed from the anterior end of the strip loin, denuded of exterior fat and connective tissue,

vacuum packaged and stored at -30°C until proximate composition was determined. Immediately anterior to the steak used for proximate analysis, five steaks, each 2.54 cm thick, were cut and vacuum packaged to assess cooking properties and tenderness. Longissimus steaks were assigned to serial aging treatments; the first of these steaks from the posterior end was cooler aged for 4d, the second for 7d, the third for 14d, the fourth for 28d, and the fifth for 56d.

Semimembranosus samples were collected after fabrication of beef rounds (IMPS 168). Muscles were faced by removing the uneven portion. A 1.3 cm thick steak was removed from the anterior end, denuded of exterior fat and connective tissue, vacuum packaged and stored at -30°C until proximate composition was determined. Immediately posterior to the steak used for proximate analysis, three steaks, each 2.54 cm thick, were cut and vacuum packaged to assess cooking properties and tenderness. Semimembranosus steaks were assigned to serial aging treatments; the first of these steaks from the posterior end was cooler aged for 7 d, the second for 14 d and the third for 56 d.

Cooler temperature was maintained at $+1°C \pm 2°C$ throughout aging periods. Aged steaks were stored at -30°C until cooking properties and tenderness characteristics were measured.

Proximate Analysis. Duplicate longissimus samples were analyzed according to procedures outlined by AOAC (1984). Samples were immersed in liquid nitrogen and subsequently powdered in a Waring® model 34BL22 commercial blender (New Hartford, CT). A 3 g powdered sample was dried at 104°C for 24 hours and cooled in a desiccator for 1 hour. Samples were weighed to determine their moisture content. Following moisture determination, samples were placed in a soxhlet and extracted for 24 hours

with petroleum ether. Samples were dried at 104°C for 12 hours, cooled in a desiccator, and weighed to determine lipid content. Crude protein was determined using the Kjeldahl method. A .5 g powdered sample was placed in digestion tubes with two Kjel® tabs (3.5 g potassium sulfate + 0.0035 g Selenium) and digested for 2 hours at 420°C. Samples were removed, extended with 75 ml of deionized water and analyzed for protein using a KJELTEC® 1030 Auto Analyzer (Hoganas, Sweden).

Steak Cookery and Shear Force. Cooking properties and shear force determinations were performed as described in AMSA (1978). Aged steaks were allowed to thaw at 3°C for 17 hours and broiled on Faberware® (Bronx, NY) open-hearth broilers to a final internal temperature of 70°C. Constantan coated copper thermocouples were placed in the geometric center of each steak. Internal temperature was monitored using an OMEGA® OM-302 temperature logger (Stamford, CT). Cooking time to a medium degree of doneness (minutes/100 g raw steak) and cooking loss (percentage weight loss) were determined for each steak. Steaks were allowed to cool to 25°C after which cores measuring 1.27 cm in diameter were removed parallel to the longitudinal axis of the muscle fibers. Individual cores were objectively measured for tenderness using the Warner-Bratzler shear apparatus attached to a Instron® Universal Testing Machine Model 4502 (Canton, MA).

Data Analysis. Animal performance and carcass characteristics were analyzed on a pen basis using least squares analysis (SAS, 1995) with a linear model that included the main effects of time on ethanol (df = 5) and weight block (df = 3). Cooking properties and shear force data were analyzed on an individual animal basis using a general linear model (SAS, 1995). Time on ethanol and age were included in the statistical model. No interactions ($P>.05$) were detected. Least squares means are reported to

account for the unequal number of steers per treatments. Additionally, linear, quadratic and cubic contrasts were utilized to compare time on ethanol least squares means. Significance is reported at the .05 level unless otherwise noted.

Results and Discussion

When feeding ethanol to feedlot cattle, two concerns were the palatability of the ration and the evaporative loss of ethanol from the diet. Thus, a preliminary study was conducted to determine the evaporative loss of ethanol from rations containing either 0% or 4.0% (DM basis) ethanol. Test rations were weighed every five minutes for the first hour, then every hour for a six hour period to determine weight loss. Test rations were held at 23°C with negligible air movement. Weight loss was not different with 0 vs. 4% added ethanol, so we concluded that evaporative loss must have been small (under 0.15%/h).

As noted in the previous chapter, feed intake tended to be reduced the first day or two that ethanol was fed. Immediately following addition of ethanol, feed became slightly cool to the touch and had a rather strong, sweet smell. This caused no persistent reduction in feed intake. Following this adaptation period, rate of feed consumption as measured by feed refusal was not numerically different between ethanol treatments. However, even though ethanol did not negatively affect feed intake, successful bunk management is an important issue to achieve consistent ethanol ingestion. All cattle were fed twice daily; on most days, all feed was consumed within 45 to 90 minutes after it was provided.

Steers fed in this trial were housed in a confinement facility. Cattle receiving ethanol appeared to be more docile during movement and were

easier to handle during weighing. The confinement environment appeared to affect steer mobility; eight steers were diagnosed with acute lameness. However, prevalence of lameness was not correlated with dietary treatments.

Steer Performance. Effects of length of ethanol supplementation on animal performance are reported in Table 2. Averaged across days fed ethanol, measures of animal performance were not altered. Initial and final weights were not different between ethanol treatments. In contrast to results of the previous short-term study with cattle nearing market weight, daily gain (live weight basis) and feed conversion (feed/gain) were not improved when ethanol was included in the diet. This might be explained partially by the manner in which ethanol was supplemented. In the previous study, as the concentration of ethanol in the diet increased (from 0 to 6.0%), calculated energy values tended to increase and cattle were limit-fed such that the higher dietary energy concentration increased ADG. This is in agreement with Larson et al. (1993) who suggested that because diets including wet distillers byproducts contain ethanol, this could account partially for the increases in NEg that they observed from these products. In contrast, calculated net energy values for ethanol treatments derived from steer performance, were similar in this study. If NEg had been increased, one would expect cattle fed ethanol longer should have been more efficient than cattle fed the control diet. However, only 4.0% ethanol was fed versus 6.0% in the previous study, and in this study, cattle had ad libitum access to feed versus a limited feed supply in the previous trial.

Limited research has been conducted to evaluate performance of cattle fed ethanol in a finishing ration. Compared with control cattle, those fed ethanol for 122d tended to have slightly better feed conversion ($P<.17$);

however, feed intake decreased linearly ($P<.05$) as ethanol was fed for more days. Ham et al. (1994) reported that addition of 5 or 10% ethanol did not affect ($P>.10$) daily gain, dry matter intake or feed efficiency of finishing steers. However, Ham and co-workers (1994) noted that as the amount of ethanol present in the diet increased, feed efficiency tended ($P=.15$) to decrease linearly. Burroughs et al. (1958) reported that ethanol supplementation at a level of only 85 g daily (about 25% the amount we fed) improved live weight gains and feed efficiency of finishing cattle fed a high concentrate diet for 134 days. Byers et al. (1979) fed Angus and Hereford x Angus steers a corn silage diet with 3% added ethanol. They reported that ADG was improved by 8.5% by added ethanol when adequate selenium was present in the diet. Interestingly, Chen et al. (1977) demonstrated that ethanol supplementation (20% v/v) had no effect on weight patterns of rats fed diets high in carbohydrates. Factors that may account for performance responses with ruminants include a possible reduction in rumen pH with added ethanol (Ham et al., 1994), a change in the microbial population (Ham et al., 1994), and increased motility and rate of passage of intestinal contents (Mezey, 1975).

Blood Analysis. Blood was analyzed for blood alcohol; concentrations were below the detection limits of the analytical method during all sampling periods. This is in contrast to the initial study in which cattle fed 4% ethanol supplementation for 14d had higher ($P<.05$) blood alcohol levels than cattle fed other concentrations of ethanol. The difference in blood alcohol concentrations may be a result of the difference in sampling times. The cattle in the previous study where sampled shortly after their p.m. feeding; cattle in this study were sampled prior to their a.m. feeding, some 15 h after being fed. This difference may have given the liver enough time to

metabolize alcohol and reduce blood concentrations below detectable levels. Furthermore, in the previous study, blood alcohol levels peaked between day 14 and day 28 and gradually declined over the remainder of the trial, a result that might reflect metabolic adaptation by the animal. However, blood alcohol levels from steers consuming the 4% ethanol diet in this study were below detection limits on d 10, 18, 32, 39 and 66 d after ethanol feeding began.

Histological and enzymatic studies have indicated that either acute or chronic ethanol ingestion causes progressive muscle injury (Song and Rubin, 1972; Kahn and Meyer, 1969; Urbano-Marquez et al., 1985). Electron microscopy data indicates that alcohol has a direct effect on the structural integrity of the myofibril (Urbano-Marquez et al., 1989). The changes that are induced by alcohol closely resemble those that occur to the myofibril during postmortem aging. Numerous studies have documented that these ethanol induced structural changes are proportional to serum creatine phosphokinase levels (Urbano-Marquez et al., 1989; Kahn and Meyer, 1969; Diamond, 1989). This is why serum creatine phosphokinase, an index of muscle damage, was measured. Results (Table 3) detected no effect of ethanol on creatine phosphokinase concentration during this feedlot study. Only on day 56 there was a hint of a difference ($P<.12$) in which creatine phosphokinase tended to be greater for steer receiving ethanol. In several studies with nonruminants, increases in serum creatine phosphokinase have been detected after chronic and acute ethanol ingestion (Rubin, 1979; Song and Rubin, 1972; Diamond, 1989). In contrast to results from the initial study, creatine phosphokinase levels were not elevated in this study either at the beginning or at the conclusion of the study. Whether this represents

differences in sampling time or some other unidentified factor that was different in the two trials is not known.

Carcass traits. Carcass characteristics were not significantly changed ($P>.05$) by time of ethanol feeding (Table 4). These results are in agreement with the initial study and with Burroughs et al. (1958). Cattle fed ethanol for only the final 17 d prior to slaughter tended to have numerically thicker subcutaneous fat and more cattle grading U.S. Choice when compared to all other treatments. However, the percentage of steers grading U.S. Choice decreased when ethanol had been fed more days. Steers not receiving ethanol in both the initial and present study had fewer cattle grading U.S. Choice (study 1 = 67%, present study = 62%) in relation to the 2% ethanol treatment in the first study (U.S. Choice = 81%) and when cattle were fed ethanol for 17d in this study (U.S. Choice = 80%).

Composition, Cooking Properties and Shear Force. Dietary treatments did not significantly alter the proximate analysis composition of longissimus samples (Table 5). Cooking shrink and cooking time results for longissimus samples are presented in Table 6. Laakonen et al. (1970) reported that degree of doneness in meat is extremely critical in affecting tenderness and cooking yield. Cooking shrink appeared to be affected in a cubic manner ($P<.10$) by days of ethanol feeding for steaks aged 4, 14, 28 and 56 d. This cubic response is difficult to explain; proximate analysis was not significantly different for any individual times of ethanol feeding. Cooking time was not changed by feeding ethanol for more days prior to slaughter.

Aging or conditioning of meat is the process of storing meat under controlled temperature and relative humidity. From a commercial viewpoint, the rate of aging and the improvement in tenderness are important aspects to profitability and to consumer satisfaction. In the meat industry, meat is

typically stored at 0 to 3°C for 7 to 14 days; however, the logistics of inventory and storage facilities may dictate otherwise. Morgan et al. (1991) found that the average post-fabrication time, defined as the time for primals and sub-primals to arrive to retail outlets, for all cuts was approximately 17 d.

Several researchers have documented that the extent of improvement from postmortem aging is dependent on the structural integrity of the myofibril. Factors that directly affect myofibrillar structural integrity include breed, electrical stimulation, muscle location, sarcomere length and induced cellular affects by ethanol. Urbano-Marquez et al. (1989) eloquently showed that ethanol has a direct effect on skeletal muscle integrity. Either acute or long term administration of ethanol affected the structural integrity of the myofibril and contractility. Additionally, ATPase activity and actomyosin complex are altered by low concentrations of ethanol such that the muscle can not develop normal tension; this limits muscle shortening (Puszkin and Rubin, 1976), perhaps due to reduced ability of the ATPase receptor sites to adequately bind calcium.

Tenderness of longissimus steaks was measured by determining Warner Bratzler shear force values over five aging periods. These results are shown in Table 7. Main effects of days fed ethanol and of aging time were significant ($P<.001$, $P<.0001$). Within an aging periods 4 and 14, diet composition significantly altered ($P<.05$) Warner Bratzler shear values. Postmortem aging improved ($P<.05$) Warner Bratzler shear values within all days fed treatments as a result of postmortem aging.

With steaks aged 4d, steers fed ethanol for 24 d were less tender than steaks from steers fed ethanol for longer or shorter times. Additionally, steers receiving dietary ethanol had higher Warner Bratzler shear values when

steaks were aged 4d. This trend was present during all aging times Significant quadratic and cubic effects were detected in shear force and percent very tender. Percent tender steaks tended (P=.10) to increase linearly as the number of days fed ethanol was increased. However, control animals had significantly more very tender steaks than either the 17 or 24 days fed treatments (73.7% vs. 40%, 20%).

Averaging across dietary treatments, steaks aged 7 days did not differ (P>.05) in shear force and percent tough and tender steaks although feeding ethanol resulted in fewer very tender steaks. One partial explanation for this observation is that the initial shear force (steaks aged 4 d) for controls was numerically less than treatments containing ethanol. Thus, the percentage of very tender steaks for controls would be expected to numerically larger. This observation was apparent until steaks were aged 14d, in which there were no differences (P>.05) in the percent very tender steaks across dietary treatment. The fact that shear force values differed with dietary treatments at day 4 of aging is interesting. By day 7, the rate of tenderness improvement for steers receiving ethanol for more than 17 days prior to slaughter was such that shear force values were not different. This suggests that the rate of aging for steers fed ethanol for 24 or more days was greater than either the control or 17 d dietary treatments. Percentage shear force improvement from day 4 to day 7 postmortem across days fed treatments were: control, 2.9%; 17 d ethanol, -0.25%; 24 d ethanol, 17.7%; 38 d ethanol, 6.6%; 66 d ethanol, 5.5%; 122 d ethanol, 4.5%. However, steers fed ethanol had more room for improvement as suggested by their higher initial (steaks aged 4 d) Warner Bratzler shear force values. Significant cubic effects were detected in shear force and percent very tender.

After 14 days of aging, steaks from control steers were more tender ($P<.05$) than steaks from steers fed ethanol for 17, 24 and 122 days. These results conflict with those in the initial study in which cattle ingesting 4% ethanol (DM basis) for 42 d were more tender at all aging times when compared to steers not receiving ethanol. Steaks from control steers were more tender ($P<.05$) than steaks from steers receiving ethanol for 17 or 24d prior to slaughter. From day 7 to day 14 postmortem, the percent shear force improvement was greater than 18% for controls, 17d and 38d ethanol treatments. Although not significantly different, 94.7% of control longissimus steaks were classified as very tender in this study at d 14 postmortem. Similar to steaks aged 7 d, significant cubic effects were detected for shear force and percent very tender.

No dietary treatment effects on shear force or percent tough, tender and very tender were detected for steaks aged for 28 or 56d. However, the percent shear force improvement from day 14 to day 28 postmortem was largest for steers ingesting ethanol for 17 and 122d (12.5% and 11.8%). The largest improvement in the shear force values from day 28 to day 56 occurred in steaks from cattle that had consumed ethanol for 24 d (18%). However, recall that this treatment was the least tender at day 4 postmortem.

Aging of steaks improved Warner Bratzler shear values across all dietary treatments. Shear force was improved significantly ($P<.05$) by day 7 of postmortem aging when cattle were fed ethanol for 24 days. For all other dietary treatments, aging was improved significantly by day 14 of aging. Longissimus steaks from 4% and 6% ethanol treatments in the first study were significantly improved after 7 days of aging when cattle had been fed ethanol for 42 d. Combining the results of the first and second trials may

indicate that ethanol accelerates the rate of postmortem tenderization; however, caution must be applied as because numerous variables contribute to temporal changes in tenderness.

One factor of concern in this study was that the animal to animal variation may have effected Warner Bratzler least square mean shear values. Table 8 displays the effects of ethanol on longissimus shear force standard deviations. Statistical analysis of standard deviations detected no effect of ethanol feeding time on animal to animal variability.

Length of time ethanol was fed did not affect proximate composition of the semimembranosus muscle (Table 9). Table 10 displays the effects of ethanol on cooking properties and shear force values of semimembranosus muscles ages 7, 14 and 28d. Cooking shrink was not affected ($P>.05$) at any aging time. However, cooking time was different ($P<.05$) when steaks were age 14 d. Cooking times tended to respond cubically when aged 14 and 28 d ($P = .06$; $P = .08$).

Shear force values for semimembranosus steaks aged for 7, 14 and 28d are presented in Table 11. Similar to longissimus steaks aged 7d, cattle fed ethanol for 28 d prior to slaughter were the least tender as indicated both by shear force and percent steaks classified as tough. Steers fed ethanol for 38 d had steaks that were significantly more tender than steaks from control steers. The tenderness of semimembranosus steaks was not statistically improved when aged for either 14 or 28 d when steers were fed ethanol. In contrast to the initial study, less than 43% of all semimembranosus steaks were considered to be tough when aged for 7 d. After 28 days of aging, more than 50% of all semimembranosus steaks had shear force values less than 3.86 kg.

Least square means of the standard deviations of steak to steak shear force variation are presented in Table 10. As with longissimus steaks, variation among semimembranosus shear force was not altered by number of days that ethanol had been fed.

Implications

Dietary ethanol neither depressed or augmented feedlot performance or carcass traits. The lack of genetic uniformity may have contributed to the shear force values. However, an interaction between time of ethanol feeding and steak aging time suggested that ethanol tended to increase the rate of postmortem aging. Nevertheless, steaks from control animals were as tender as steaks from steers fed ethanol for various time periods. Clearly, further research is required to understand the cellular effects of ethanol in relation to altering skeletal muscle in ruminants.

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Table 1. Composition of diets (dry matter basis)

	Ethanol (%)	
Ingredient (%)	0	4
Corn, rolled	79.27	75.98
Alfalfa hay, pelleted	4.00	3.83
Cottonseed hulls	4.98	4.78
Pelleted supplement[a]	7.47	7.16
Molasses, cane	4.27	4.09
Ethanol[b]	0	4.15
Calculated composition		
NEm, Mcal\kg[c]	2.07	2.14
NEg, Mcal\kg[c]	1.32	1.38
Crude protein, %	12.25	11.72
K, %	0.70	0.67
Ca, %	0.55	0.52
P, %	0.34	0.32

[a] Supplement composition (%): Cottonseed meal, 75.92; calcium carbonate, 12.05; urea, 5.69; salt, 4.23; dicalcium phosphate, 1.18; Rumensin®, 0.27; vitamin E, 0.21; Tylan®, 0.17; vitamin A, 0.15; trace mineral pre-mix, 0.13.

[b] Ethanol mixed into ration was 90% pure.

[c] Ethanol provides 5.30 kcal NEm\kg and 3.94 kcal NEg\kg.

Table 2. Effects of ethanol on performance of feedlot steers[a]

	Days fed ethanol[b]						Observed significance (P <)			
	0	17	24	38	66	122	SEM	Linear	Quad	Cubic
Number of steers	21	21	21	21	21	21				
Initial weight, kg	284.8	285.1	284.8	285.5	284.8	286.2	0.66	0.23	0.56	0.49
Final weight, kg	503.4	498.2	510.1	502.6	493.9	507.0	7.74	0.97	0.90	0.47
Daily gain[c], kg										
Period 1, kg	2.09	1.91	2.04	1.81	1.84	1.94	0.14	0.34	0.46	0.73
Period 2, kg	1.71	1.49	1.76	1.62	1.64	1.73	0.09	0.55	0.47	0.74
Period 3, kg	1.50	1.50	1.57	1.52	1.48	1.56	0.09	0.81	0.95	0.63
Period 4, kg	1.32	1.32	1.32	1.19	1.23	1.27	0.11	0.48	0.77	0.55
Period 5, kg	0.39	0.36	0.92	1.06	0.51	0.77	0.24	0.25	0.22	0.92
Overall, kg	1.48	1.44	1.52	1.47	1.42	1.49	0.05	0.89	0.94	0.51
Feed intake, kg	9.05	8.91	9.04	8.94	8.75	8.63	0.14	0.05	0.38	0.73
Feed\gain	6.15	6.19	5.95	6.12	6.20	5.82	0.17	0.31	0.60	0.30
ME, Mcal\kg DM	3.02	3.01	3.09	3.03	3.00	3.15	0.06	0.23	0.49	0.26
NEm, Mcal\kg DM	1.98	1.97	2.05	2.00	1.97	2.12	0.06	0.23	0.48	0.24
NEg, Mcal\kg DM	1.32	1.31	1.36	1.33	1.31	1.40	0.03	0.24	0.51	0.28

[a] Least square means.

[b] Number of days in which ethanol was fed prior to slaughter.

[c] Period 1-4 = 28 d periods; Period 5 = 8 d period.

Table 3. Effects of ethanol on creatine phosphokinase (CPK) levels of feedlot steers[a]

	Days fed ethanol[b]						Observed significance (P <)		
	0	17	24	38	66	122	SEM	Linear	Quad	Cubic
Number of steers	21	21	21	21	21	21				
CPK, IU\L blood										
Day 1	139.71	139.95	125.90	125.43	179.90	131.29	24.47	0.71	0.89	0.33
Day 56	172.43	174.67	148.24	168.90	135.24	148.57	16.73	0.12	0.86	0.74
Day 120	133.48	145.48	135.29	130.24	136.62	118.00	11.69	0.27	0.42	0.98

[a] Least square means.

[b] Number of days in which ethanol was fed prior to slaughter.

Table 4. Effects of ethanol on carcass characteristics of feedlot steers[a]

	Days fed ethanol[b]						Observed significance (P <)			
	0	17	24	38	66	122	SEM	Linear	Quad	Cubic
Number of steers	21	21	21	21	21	21				
Carcass weight, kg	328.3	322.3	326.4	325.3	321.4	330.0	5.08	0.92	0.40	0.78
Dressing percentage	62.6	62.2	61.5	62.1	62.4	62.5	0.49	0.81	0.18	0.46
Fat thickness, cm	1.0	1.3	1.0	1.0	1.1	1.1	0.13	0.88	0.73	0.33
Ribeye area, cm^2	83.2	78.5	82.5	80.5	79.2	82.6	1.87	0.85	0.30	0.97
KPH fat, %	2.3	2.1	2.0	2.3	2.1	2.4	0.17	0.64	0.12	0.83
USDA yield grade	2.6	3.0	2.6	2.7	2.9	2.8	0.18	0.75	0.79	0.51
Percent yield grade 4	4.8	4.8	9.5	4.8	9.5	9.5	6.21	0.54	1.0	0.91
Maturity score[c]										
Skeletal	140	148	139	139	145	143	2.6	0.72	0.53	0.32
Lean	133	135	137	138	139	135	2.2	0.26	0.17	0.53
Overall	137	141	138	138	142	139	2.2	0.44	0.74	0.77
Marbling score[d]	430	451	434	399	425	422	23.3	0.46	0.82	0.39
Percent Choice	61.9	81.0	66.7	57.1	57.1	57.1	11.8	0.31	0.73	0.28
Condemned liver, %	14.3	23.8	9.5	9.5	23.8	9.5	7.9	0.72	0.95	0.82

[a] Least square means.

[b] Number of days in which ethanol was fed prior to slaughter.

[c] Maturity score: 100-199 = A, approximately 9-30 months of age.

[d] Marbling score: 400 = Small 00, minimum for USDA Choice.

Table 5. Effects of ethanol on Longissimus proximate composition[a]

	Days fed ethanol[b]						Observed significance (P <)		
	0	17	24	38	66	122	SEM	Linear	Quad	Cubic
Number of steers	19	20	20	21	20	20				
Protein, %	22.3	21.8	22.5	22.2	21.8	22.2	0.19	0.58	0.96	0.80
Moisture, %	72.8	72.7	72.5	72.7	72.2	73.0	0.32	0.87	0.28	0.39
Lipid, %	3.90	4.58	3.96	3.45	4.47	3.67	0.35	0.50	0.81	0.72

[a] Least square means.

[b] Number of days in which ethanol was fed prior to slaughter.

Table 6. Effects of ethanol on Longissimus cooking properties[a]

	Days fed ethanol[b]						Observed significance (P <)			
	0	17	24	38	66	122	SEM	Linear	Quad	Cubic
Number of steers	19	20	20	21	20	20				
Aged 4 days										
Cooking shrink, %	28.36	29.96	30.36	29.48	29.70	30.08	0.59	0.17	0.21	0.08
Cooking time[c]	8.26	8.80	8.71	8.29	8.77	8.15	0.26	0.62	0.14	0.72
Aged 7 days										
Cooking shrink, %	28.75	29.71	29.22	29.75	29.64	29.13	0.48	0.59	0.19	0.96
Cooking time	8.75	9.18	8.50	9.38	9.25	8.93	0.26	0.38	0.53	0.38
Aged 14 days										
Cooking shrink, %	28.22	29.86	29.83	29.43	29.35	30.13	0.46	0.05	0.28	0.02
Cooking time	8.85	8.94	8.84	8.79	8.75	8.70	0.25	0.52	0.85	0.81
Aged 28 days										
Cooking shrink, %	28.50	30.84	29.73	29.94	29.02	29.70	0.68	0.89	0.23	0.05
Cooking time	8.86	9.38	9.07	9.26	9.11	9.24	0.25	0.55	0.56	0.37
Aged 56 days										
Cooking shrink, %	30.99	32.33	31.83	31.68	31.17	31.65	0.54	0.94	0.39	0.10
Cooking time	8.98	9.27	9.23	9.40	9.09	8.99	0.25	0.89	0.19	0.86

[a] Least square means.

[b] Number of days in which ethanol was fed prior to slaughter.

[c] Cooking time expressed as minutes to 70°C\100 g raw steak.

Table 7. Effects of ethanol on Longissimus shear force values[a]

	Days fed ethanol[b]							Observed significance (P <)		
	0	17	24	38	66	122	SEM	Linear	Quad	Cubic
Number of steers	19	20	20	21	20	20				
Aged 4 days										
Shear force, kg	3.52^{dh}	4.04^{dh}	4.85^{ch}	4.06^{dh}	3.63^{dh}	3.97^{dh}	0.2	0.90	0.01	0.01
Tough[l], %	21.1^{de}	25.0^{cde}	50.0^{c}	38.1^{cd}	5.0^{e}	25.0^{cde}		0.53	0.09	0.12
Tender[m], %	5.3^{f}	35.0^{cde}	30.0^{cdef}	9.5^{ef}	45.0^{c}	30.0^{cdef}		0.10	0.48	0.29
Very tender[n], %	73.7^{c}	40.0^{de}	20.0^{e}	52.4^{cd}	50.0^{cd}	45.0^{cde}		0.38	0.03	0.02
Aged 7 days										
Shear force, kg	3.42^{h}	4.05^{h}	4.12^{i}	3.81^{h}	3.44^{h}	3.80^{hi}	0.2	0.88	0.09	0.01
Tough, %	10.5	25.0	26.3	14.3	10.0	15.0		0.63	0.37	0.13
Tender, %	5.3	40.0	21.1	33.3	20.0	35.0		0.22	0.40	0.07
Very tender, %	84.2^{c}	35.0^{e}	52.6^{de}	52.4^{de}	70.0^{cd}	50.0^{de}		0.47	0.15	0.01
Aged 14 days										
Shear force, kg	2.89^{ei}	3.43^{cdi}	3.70^{cij}	3.22^{dei}	3.01^{dei}	3.40^{cdi}	0.1	0.59	0.10	0.002
Tough, %	0	5.0	15.0	4.8	0	5.0		0.99	0.19	0.13
Tender, %	5.3	25.0	20.0	14.3	15.0	20.0		0.60	0.51	0.14
Very tender, %	94.7	70.0	65.0	81.0	85.0	75.0		0.63	0.20	0.03
Aged 28 days										
Shear force, kg	2.81^{i}	3.05^{ij}	3.35^{jk}	2.93^{i}	2.90^{i}	3.04^{j}	0.1	0.81	0.15	0.04
Tough, %	0	0	10.0	0	0	0		0.67	0.12	0.29
Tender, %	5.3	10.0	5.0	0	0	5.0		0.34	0.61	0.15
Very tender, %	94.7	90.0	85.0	100	100	95.0		0.30	0.70	0.07

Aged 56 days

Shear force, kg	2.75[i]	2.87[j]	2.84[k]	2.80[i]	2.76[i]	2.97[j]	0.1	0.45	0.71	0.21
Tough, %	0	0	0	0	0	5.0		0.15	0.19	0.37
Tender, %	10.5	5.0	0	0	5.3	0		0.13	0.26	0.32
Very tender, %	89.5	95.0	100	100	94.7	95.0		0.49	0.11	0.63

a Least square means.

b Number of days in which ethanol was fed prior to slaughter.

c,d,e,f Means in the same row with a common superscript letter are not different ($P > .05$).

h,i,j,k Shear force means in the same column with a common superscript letter are not different ($P > .05$).

l Percentage of steaks with shear force values of 4.54 kg or higher.

m Percentage of steaks with shear force values between 3.86 and 4.53 kg.

n Percentage of steaks with shear force values less than 3.86 kg.

Table 8. Effects of ethanol on Longissimus shear force standard deviations[a]

	Days fed ethanol[b]						Observed significance (P <)		
	0	17	24	38	66	122	SEM	Linear	Quad	Cubic
Number of steers	19	20	20	21	20	20				
Standard deviation										
Aged 4 days	0.77	0.72	0.85	0.72	0.80	0.68	0.07	0.57	0.43	0.62
Aged 7 days	0.80	0.69	0.77	0.73	0.68	0.79	0.07	0.89	0.41	0.88
Aged 14 days	0.62	0.65	0.69	0.62	0.60	0.72	0.07	0.63	0.70	0.21
Aged 28 days	0.69	0.76	0.65	0.64	0.62	0.68	0.06	0.34	0.52	0.25
Aged 56 days	0.58	0.64	0.58	0.65	0.58	0.67	0.06	0.50	0.91	0.44

[a] Least square means.

[b] Number of days in which ethanol was fed prior to slaughter.

Table 9. Effects of ethanol on Semimembranosus proximate composition[a]

	Days fed ethanol[b]						Observed significance (P <)		
	0	17	24	38	66	122	SEM	Linear	Quad	Cubic
Number of steers	21	21	21	21	21	19				
Protein, %	22.0	22.2	22.1	22.2	22.2	22.2	0.18	0.41	0.73	0.74
Moisture, %	75.3	74.6	74.5	74.8	74.8	74.8	0.21	0.50	0.08	0.08
Lipid, %	1.5	2.1	2.0	1.6	2.0	1.8	0.20	0.85	0.35	0.14

[a] Least square means.

[b] Number of days in which ethanol was fed prior to slaughter.

Table 10. Effects of ethanol on Semimembranosus cooking properties[a]

	Days fed ethanol[b]							Observed significance (P <)		
	0	17	24	38	66	122	SEM	Linear	Quad	Cubic
Number of steers	21	21	21	21	21	19				
Aged 7 days										
Cooking shrink, %	34.47	33.73	34.35	34.10	32.95	37.26	1.39	0.34	0.16	0.28
Cooking time[c]	8.06	8.33	8.08	8.37	7.48	7.68	0.33	0.13	0.33	0.52
Aged 14 days										
Cooking shrink, %	33.43	32.36	33.73	33.26	33.39	32.70	0.91	0.89	0.72	0.47
Cooking time	6.79[e]	7.46[d]	6.98[de]	7.30[d]	6.56[f]	6.87[e]	0.21	0.25	0.14	0.06
Aged 28 days										
Cooking shrink, %	32.84	34.44	33.57	34.77	33.32	33.44	0.75	0.90	0.16	0.55
Cooking time	6.41	6.81	6.38	6.67	6.21	6.78	0.20	0.86	0.71	0.08

[a] Least square means.

[b] Number of days in which ethanol was fed prior to slaughter.

[c] Cooking time expressed as minutes to 70°C\100 g raw steak.

[d,e,f] Means in the same row with a common superscript letter are not different (P > .05).

Table 11. Effects of ethanol on Semimembranosus shear force values[a]

	Days fed ethanol[b]							Observed significance (P <)		
	0	17	24	38	66	122	SEM	Linear	Quad	Cubic
Number of steers	21	21	21	21	21	19				
Aged 7 days										
Shear force, kg	4.27[cd]	4.60[c]	4.54[cd]	3.97[e]	4.10[de]	4.09[de]	0.16	0.03	0.53	0.03
Tough[h], %	38.1	42.9	47.6	14.3	28.6	21.1		0.06	0.80	0.28
Tender[i], %	33.3	38.1	28.6	33.3	47.6	47.4		0.26	0.48	0.91
Very tender[j], %	28.6	19.0	23.8	52.4	28.6	31.6		0.40	0.58	0.22
Aged 14 days										
Shear force, kg	3.94	4.15	4.19[fg]	3.92	3.72	3.80	0.17	0.11	0.30	0.14
Tough, %	9.5	33.3	28.6	19.0	9.5	5.3		0.15	0.04	0.10
Tender, %	47.6	28.6	38.1	19.0	38.1	31.6		0.43	0.30	0.62
Very tender, %	42.9	38.1	33.3	61.9	52.4	63.2		0.06	0.56	0.44
Aged 28 days										
Shear force, kg	3.86	3.94	3.78[g]	3.63	3.60	3.72	0.14	0.12	0.60	0.21
Tough, %	9.5	19.0	9.5	4.8	0	10.5		0.29	0.68	0.06
Tender, %	28.6	28.6	33.3	19.0	28.6	31.6		0.99	0.72	0.59
Very tender, %	61.9	52.4	57.1	76.2	71.4	57.9		0.54	0.56	0.11

[a] Least square means.

[b] Number of days in which ethanol was fed prior to slaughter.

[c,d,e] Means in the same row with a common superscript letter are not different ($P > .05$).

[f,g] Shear force means in the same column with a common superscript letter are not different ($P > .05$).

[h] Percentage of steaks with shear force values of 4.54 kg or higher.

[i] Percentage of steaks with shear force values between 3.86 and 4.53 kg.

[j] Percentage of steaks with shear force values less than 3.86 kg.

Table 12. Effects of ethanol on Semimembranosus shear force standard deviations[a]

	Days fed ethanol[b]						Observed significance (P <)		
	0	17	24	38	66	122	SEM	Linear	Quad	Cubic
Number of steers	21	21	21	21	21	19				
Standard deviation										
Aged 7 days	1.01	0.94	0.86	0.89	0.95	0.93	0.08	0.65	0.24	0.55
Aged 14 days	0.86	0.81	0.75	0.99	0.77	0.86	0.09	0.83	0.93	0.59
Aged 28 days	0.60	0.69	0.60	0.72	0.59	0.59	0.07	0.72	0.39	0.83

[a] Least square means.

[b] Number of days in which ethanol was fed prior to slaughter.

CHAPTER V

SUMMARY OF THE EFFECTS OF FEEDING ETHANOL ON PERFORMANCE OF FEEDLOT STEERS, CARCASS CHARACTERISTICS AND MEAT TENDERNESS

PREFACE

The purpose of this chapter is two fold. Initially, I intend to summarize the results and limitations of the two ethanol feeding trials which we conducted. Secondly, I will present several areas for research and utilization of basic techniques to elucidate the mechanism(s) involved in meat tenderness.

INTRODUCTION

Product consistency of beef needs to be improved (National Beef Tenderness Conference, 1994). Despite widespread use of electrical stimulation and postmortem aging, data from the National Beef Tenderness Survey (Morgan et al., 1991) indicated that the coefficient of variation in shear force (26 retail cuts sampled) of the US fed-beef slaughter population was over 21%. This has caused the beef industry to renew its emphasis in developing antemortem and postmortem systems to improve both tenderness and the consistency of tenderness. Although the relationship between tenderness and the highly dynamic nature of pre-rigor muscle has been studied extensively, much remains to be done before the research can be applied to improve consumer satisfaction.

Our research was an attempt to apply evidence that ethanol imparts acute and long-term changes to skeletal muscle (Urbano-Marquez et al., 1989). By modifying the permeability of the sarcolemma, ethanol alters contractile activity (Rubin et al., 1976; Puszkin and Rubin, 1976; and

Urbano-Marquez et al., 1989). Maintaining muscle fibers in a non-contractile condition can avoid muscle shortening and thereby enhance meat tenderness. Histological and enzymatic studies indicate that both acute and chronic ethanol ingestion causes myopathy (Song and Rubin, 1972; Kahn and Meyer, 1969; Urbano-Marquez et al., 1985) that closely resembles postmortem aging. In two studies, we examined the effect of ethanol on feedlot performance, carcass characteristics, and, in particular, meat tenderness.

Data Comparisons of Ethanol Trials 1 and 2

Experimental Design. Initially, a small feeding study was designed to examine effects of several different levels of dietary ethanol effect on steer performance and meat tenderness. Crossbred steers (N = 24; initial weight = 472 kg) were limit fed a 91% concentrate diet supplemented with ethanol added at either 0, 2, 4 or 6% of diet DM in a completely randomized design. Animal performance, carcass and palatability characteristics were analyzed using individual animals as experimental units in a 42 d feeding study.

Based on results of the initial study, a second study was developed to examine whether duration of ethanol consumption prior to slaughter would alter feedlot characteristics and palatability traits. Steers (n = 126; 286 kg) were fed a high concentrate diet; this diet was supplemented with 4% of the diet DM as ethanol for either 0, 17, 24, 38, 66 or 122 days prior to slaughter. Animal performance and carcass characteristics were analyzed on a pen basis. Cooking properties and shear force data were analyzed on an individual animal basis.

Ethanol Trial Results. Results from the initial study indicated that rate and efficiency of gain tended to be improved slightly by added ethanol. In contrast, daily gain and feed conversion for steers in the second trial were

not improved by including ethanol in the diet. This might be explained partially by the manner in which feed and ethanol were provided. First, In the initial study, cattle were limit-fed so that an increased concentration of energy would increase daily gain. In contrast, steers had ad libitum access to feed in the second study. Secondly, only one level of ethanol (4%) was fed in the second study for various time period versus a range of 0 to 6% in the first study. This altered the statistical approach and power of the experiment. Additionally, the second study only includes analyses for linear, quadratic and cubic effects of time. It is suggested that similar orthogonal contrasts be performed for control versus all ethanol treatments in the second study. Third, individual animals were used as the experimental unit, perhaps inappropriately from a statistical viewpoint, whereas a more appropriate error term, pens of cattle, were considered the experimental unit in the second study. Fourth, with limit feeding in the first trial, time for volatilization of the ethanol added to the feed was very short; in contrast, with ad libitum access to feed in the second trial, more of the ethanol probably volatilized prior to being consumed by the steers. Both trials were conducted in Stillwater, Oklahoma from November through March, however, feed bunks in the first trial were exposed to the elements while feed bunks in the second trial were part of a confinement facility.

Serum was collected to determine blood alcohol and creatine phosphokinase concentrations. Responses were detected for cattle in the initial study which were sampled shortly after their p.m. feeding; cattle in the second study were sampled prior to their a.m. feeding, some 15 h after being provided their ethanol. A longer postprandial time gives the ruminal microbes and liver more time to metabolize consumed alcohol and reduce blood concentrations below detectable levels. In the initial study, blood alcohol

levels peaked between day 14 and day 28 and gradually declined over the remainder of the trial. That may reflect metabolic adaptation by the animal. Nevertheless, blood alcohol concentrations of steers that consumed diets containing 4% ethanol diet in the second trial were below detection limits on d 10, 18, 32, 39 and 66 d after ethanol feeding began. The difference in sampling times may explain differences in blood alcohol concentrations between studies. More frequent feeding might increase the persistency of metabolic alterations and enhance effects of ethanol on muscular tissue.

In the first study, creatine phosphokinase, an index of muscle damage, peaked between 14 and 28 days of ethanol consumption but declined thereafter. However, in the second trial, we detected no effect of duration of ethanol feeding on creatine phosphokinase concentrations. However, on day 56 of the second trial, there was a hint of a linear effect ($P<.12$) in which creatine phosphokinase tended to be greater for steers receiving ethanol. Perhaps, as with ethanol discussed above, differences may be due to differences in postprandial sampling time.

Carcass measurements including abscess incidence were not affected in either trial. Although not statistically different, the percentage of carcasses attaining the US Choice grade in the first trial tended to decrease when more ethanol was present in the diet. Similarly, the percentage of carcasses grading US Choice decreased when steers were fed ethanol for longer periods of time. Although several studies have indicated that ethanol may alter plasma free fatty acid concentrations and turnover of free fatty acids, whether ethanol might directly or indirectly alter metabolism of intramuscular fat is not known.

Ethanol supplementation (both level and days fed trials) enhanced the rate of tenderness improvement during aging but failed to significantly

improve tenderness at any specific aging time. In the initial trial, longissimus muscle aging effects became significant after 28 d for controls, 14 d for steers fed 2% ethanol and 7d for steers receiving 4 or 6% ethanol. The rate of increase in tenderness of the longissimus muscle was no greater with 6% than with 4% dietary ethanol. In the second study, significant improvements in rate of increase in tenderness were detected by day 14 postmortem for steers fed ethanol for 0, 17, 38, 66 and 122 days prior to slaughter. However, when ethanol had been consumed for 24 d prior to slaughter, rate of increase in tenderness was observed with less aging time (7 days of aging). In the second trial, initial shear force (steaks aged 4 d) was numerically less for control steers than for steers fed the diet containing ethanol. Thus, steers fed ethanol had more room for tenderness to improve. Combining the results of the first and second trials indicate that ethanol accelerates the rate of postmortem tenderization; however, caution must be applied as because numerous factors can contribute to temporal changes in tenderness.

Future research: Collectively, this research provided insight to some questions but left others unanswered. Apparently, ethanol supplemented as 4% of the diet DM did not alter feedlot performance or carcass characteristics markedly. Metabolism in the rumen deserves deeper study. Ethanol may have altered ruminal pH, the microfloral population, volatile fatty acid production, and(or) metabolite absorption and metabolism to alter muscle composition or metabolism. Further study could reveal if ethanol-induced myopathy is reversible. Also, does the type of diet in which ethanol is included (roughage vs. concentrate) alter effects of ethanol on meat palatability?

Present research suggests that ethanol may effect skeletal muscle when cattle are fed a high concentrate ration. However, caution is advised when interpreting this data. Cell physiology studies are required to determine: 1). Did ethanol cross the sarcolemma? 2). If ethanol has a cellular effect on ruminant skeletal muscle, does the magnitude of its effect vary with rumen function and circulating blood ethanol levels? 3). Further research is needed to elucidate the mechanism by which ethanol effects meat tenderness.

To determine the mechanism by which ethanol effects meat tenderness, physiological studies could use skinned muscle fibers. Contractile force could be measured while the chemical environment of the preparation is altered precisely. Each component of muscle contraction could be isolated and observed. Length-tension curves from such research should be applicable to length changes that occur postmortem which ultimately determine meat tenderness. Initially, the question could asked, does ethanol limit the ability of the muscle to contract? If so, how does ethanol affect the structural integrity of myofibrillar proteins or regulate contraction? Does ethanol interfere with myosin ATPase functionality or possibly by decreasing Calcium binding to tropomyosin?

Another very practical question is, at a constant time postmortem, why is rate of tenderness improvement increased despite a tendency for a decrease in the percent US Choice? Is the current system of classifying beef palatability after various periods of aging based on marbling obsolete or at best of limited accuracy? Considering the window of consumer acceptance relative to beef palatability and postmortem aging, might ethanol-fed steers be more likely to meet consumer demands for greater tenderness but reduced caloric content? A faster rate of aging translates to improvements in

consistency at the retail counter and less marbling may result in a product possessing less calories.

The above comments and future research ideas are based on results of the two research trials conducted. Both studies are limited when one considers the total size and diversity of the cattle population. Further, some would question using individual animal as the experimental unit in the first study. Since the smallest unit on which we could impose a treatment was the pen of cattle, is it correct to analyze individual animal data? However, the initial study essentially was a preliminary trial whose objective was to test findings that appeared in the medical community. Likewise in the second trial, although no treatment by weight block interaction was detected as being significant, treatments were only replicated across, not within, weight blocks. Although blocks were utilized to account for weight differences between steers, presence of a weight by ethanol interaction would diminish the statistical power of detecting the main effect of ethanol.

I would like to conclude this chapter with some advice from my father. No matter the knowledge you desire, you must first construct a list of questions that you are seeking to answer. Second, use statistics to design a study capable of yielding inference to these questions. Third, remember that results may be relevant only under the conditions of the study. Finally, whether the results support the initial hypothesis our not, publish the results in a timely fashion because studies that "re-invent the wheel" are inefficient and unproductive. Agriculturists often are hesitant to change. I hope that my effort and dissertation will encourage others to try new ideas that may solve problems in our industry. It is humbling to conclude that further studies involving ethanol are needed. Continued support of basic and applied

research in the area of meat quality and tenderness is imperative if we are to satisfy the palate of the American consumer.

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Ode of the Soused Steer - Anonymous

If you serve them dry martini,
and you play Henry Mancini
to your steers before you take them to the fair;
You'll be judged the Great Houdini,
when their steaks shear like a weenie,
if of ethanol each steer drank its full share.
If your meat is not so tender,
you can put it in a blender,
you can shock it, age it, blast it, eat it rare.
When your steers go on a bender,
irrespective of their gender,
you must label steaks - "Teetotalers Beware."

VITA

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