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POST-EXSANGUINATION INFUSION OF BOVINE AND OVINE CARCASSES: EFFECT ON POSTMORTEM METABOLIC CHANGES AND THE QUALITY OF MEAT

By

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ABSTRACT

POST-EXSANGUINATION INFUSION OF BOVINE AND OVINE CARCASSES: EFFECT ON POSTMORTEM METABOLIC CHANGES AND THE QUALITY OF MEAT

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MUSTAFA MOHAMMED FAROUK

This study addressed the following: 1) could the infusion of solution composed of .23% dextrose, .21% glycerin, .14% phosphate blend and .1% maltose improve tenderness, 2) how is the composition of the muscles in the infused carcasses likely to be affected, 3) how would the process affect the functional properties of meat and 4) if indeed tenderness is improved, what would be the probable causes for the improvement in the tenderness? Ninety cows were divided into two treatment groups: Control (Ctr) and infused (Inf) with the tenderizing solution listed above. Twenty four lambs were divided into 3 treatment groups: 1) Ctr, 2) infused with the same volume by weight of the same solution as the cows (Nca) and 3) Nca plus .015M $CaCl_2$ (Wca). Shear force measurement indicated that when compared to Ctr, infusion without Ca^{2+} improved tenderness significantly ($P< .05$) by 13% and 27% in Beef and lamb loin muscles respectively. However, infusion with .015M $CaCl_2$ caused increased toughness in lamb loins compared to Ctr. The result of myofibrillar fragmentation index (MFI) measurement corroborated what was observed with shear force measurement. The distribution of solution in the infused carcasses was not uniform. The amount of solution retained was in the order:

chuck/shoulder > loin > round/leg. Results of Hunter L, a & b indicated that in freshly cut lambs leg steak before storage, redness (a) in the samples was in the order: Nca > Ctr > Wca, but after storage (Refrigerated and Frozen) for 1 wk, 'a' value was in the order: Ctr > Nca > Wca. Lightness (L) and yellowness (b) were increased in the steaks as a result of infusion. Water-holding capacity (WHC) as determined by drip, thaw drip and cook losses was not affected by infusion in refrigerated samples, but WHC was lowered in frozen ones. Protein extractability was improved in beef due to infusion. Increased proteolytic activities was evidenced by the appearance of 22 to 30 KD protein components in the SDS-PAGE pattern of Nca samples. This is suported by the earlier completion of glycolitic changes as measured by the change in pH, ATP and lactic acid concentrations in these samples. Using the scanning electron microscopy, There was evidence of more fragmentation, fracturing and the splitting of myofibrills of Nca. These are probable reasons for the observed greater tenderness in the samples. Calcium ion induced toughening was the reason for the lower tenderness observed in Wca samples. Wca treatment was detrimental to the color and WHC of meat.

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LITERATURE REVIEW

Postmortem Metabolic Changes in Conversion of Muscles to Meat

Many features of meat that are essential from the consumer point of view depend on the processes involved in the conversion of energy-replete muscle to energy-free meat (Jeacocke, 1984, Lee et al., 1978). Marsh (1981) reported that water-holding capacity (WHC), tenderness, emulsification, color, bacterial control, juiciness, fermentation and aging are some of the features that are dependent on the nature, extent and the rate of metabolic change in the first few hours following slaughter.

When an animal is slaughtered, the heart stops beating; therefore, the circulatory system ceases to supply the muscle with oxygen and glucose (Davey, 1984; Pearson, 1987; Honikel and Fisher, 1977; Hamm, 1977). It is the breakdown of this glucose in the presence of oxygen that provides most of the energy (ATP) utilized by muscle in the living animal. Therefore, in the absence of this metabolizable fuel, skeletal muscles subsequently have to rely on anaerobic metabolism of their intracellular carbohydrate stores (Jeacocke, 1984). According to Pearson (1987) and Bodwell et al. (1965), glycogen is the only source of energy under these postmortem conditions. Through the process of glycolysis, glycogen is then anaerobically broken down to obtain ATP (Pearson and

Dutson, 1985; Marsh, 1981). For every hexose unit of the glycogen broken through the process of glycolysis, 3-ATP molecules and 1 mol of proton (H^+) per lactate are generated (Jeacocke, 1984; Bendall, 1973). Bodwell et al. (1965) reported the initial level of glycogen in beef immediately after slaughter to be about 56.7 uMoles/g tissue. About 5-10 um ATP/g and 13.1 uMoles lactate/g tissue were reported to exist in muscles immediately after slaughter (Hamm, 1982; Bodwell et al., 1965). A summary of an aerobic breakdown of muscle glycogen is shown in Figure 1.

ATP in muscle is used to maintain contractile activity which is defined as the relative sliding of the thick and thin filament in the sarcomere of muscle myofibril (Davey, 1984). As ATP is used up by muscle in contractile activity, its hydrolysis is compensated by further breakdown of glycogen and also by the creatine kinase regeneration of ATP from creatine phosphate (Jeacocke, 1984; Bendall, 1951). Bendall (1951) reported that creatine phosphate will maintain the level of ATP until the amount is reduced to about 30% of its initial level before there is any net loss of ATP. Stored glycogen will also continue to be broken down until it is exhausted. As a consequence of this, lactate will continue to accumulate resulting in pH decline (Pearson, 1987; Marsh, 1981; Winger et al., 1979). At about pH of of 5.9-6.3 and ATP level of 1 uMoles ATP/g,

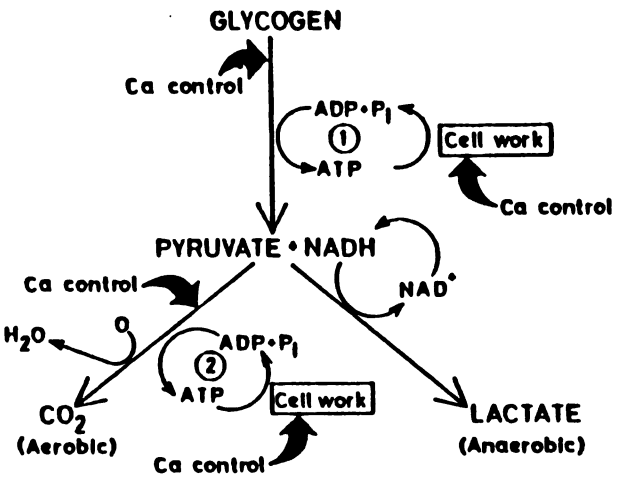


Figure 1 Aerobic and anaerobic metabolism of muscle glycogen. ATP resynthesis from ADP plus inorganic phosphate (P_i) takes place by substrate level phosphorylaton (1) and by oxidative phosphorylation (2). In addition, ATP resynthesis can occur (3) at the expense of the conversion of creatine phosphate (CrP) to creatine (Cr). This latter reaction is catalysed by the enzyme creatine kinase and is near equilibrium in the resting cell. The points at which Ca^{2+} ions exert control over the metabolic pathways are indicated. (Jeacocke, 1984).

the muscle begins to stiffen signaling the onset of rigor mortis (Honikel et al., 1981a; 1983; Bendall, 1978; Tarrant and Mothersill, 1977). Development of rigor mortis takes place in two stages. The early (delay) phase is characterized by availability of ATP, free sliding of thick and thin filaments and extensibility of the muscle. The rapid phase is marked by linear fall in ATP concentration, establishment of cross bridges between thin and thick filaments and finally with the complete disappearance of ATP, crossbridging is completed and the muscle enters the state of rigor mortis, and in so doing is has been converted to meat (Davey, 1984; Jolley et al., 1981; Forrest et al., 1975). The relationship between pH, ATP, creatine phosphate (PC) and muscle extensibility during rigor development is depicted in Figure 2. Honikel et al. (1983) indicated that completion of rigor in neck muscle was reached between 10°C and 38°C at pH 5.5-5.6 and when less than 0.5 uMoles ATP/g tissue remained. The time it takes muscle from a well fed and rested animal with a normal initial postmortem pH of 7.3-6.9 to drop to pH 5.5-5.7 (ultimate pH) ranges between 8 and 24 hours (Hamm, 1977; Bodwell et al., 1965; Bendall, 1973; 1951; Bate-Smith and Bendall, 1949). In rabbit psoas muscles, the time after death at which the onset of rigor begins depended on the magnitude of glycogen content which also determines the pH at the onset of rigor (Batesmith and Bendall, 1949). Marsh (1954) stated that the time of the

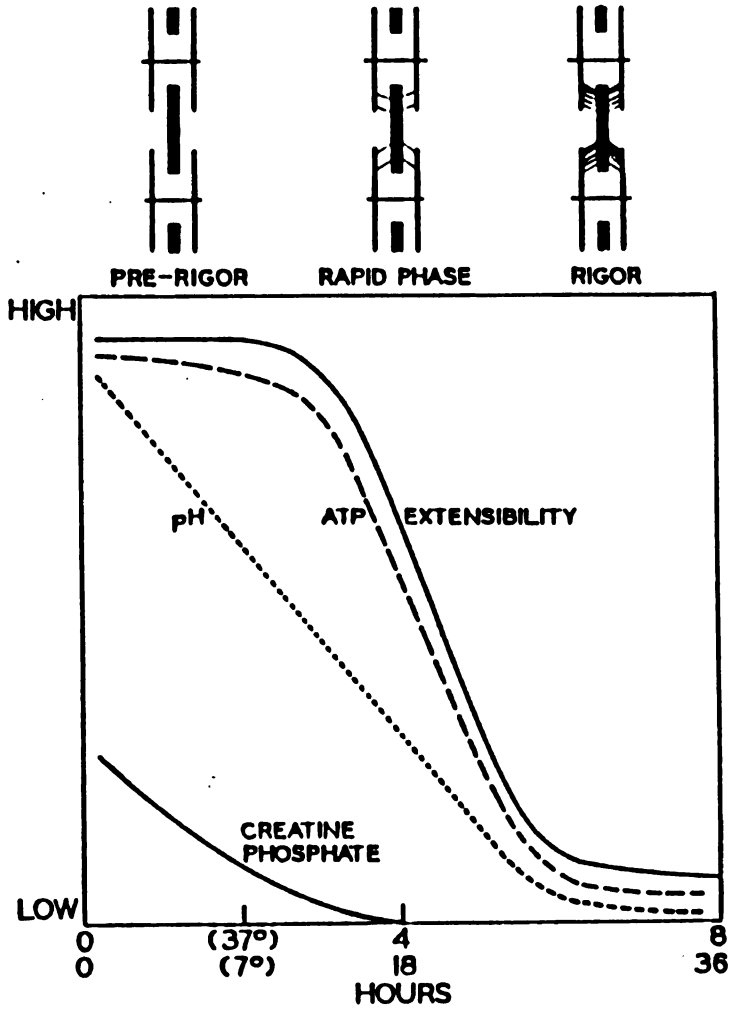


Figure 2. Rigor onset in beef at 37°C and 7°C, and cross-bridge formation between thick and thin filaments as rigor develops. (Marsh, 1981).

completion of the onset of rigor varies according to chiller efficiency, initial pH and ultimate pH.

The rate and extent of post-mortem changes (glycolysis) are affected by several factors. According to Lawrie (1985), some of the factors affecting the rate and extent of postmortem pH fall include intrinsic factors (such as species, type of muscles and variability between animals) and extrinsic factors (such as the administration of drugs pre-slaughter and environmental temperature). A summary of several studies reported by Tarrant and Mothersill (1977) indicated that the rate of post-mortem glycolysis was influenced by temperature, muscle fiber type, secretion of hormones and intensity of nervous stimuli reaching the muscle before and during slaughter. Bate-Smith and Bendall (1949) did not find any difference in the rate of change in pH from animal to animal in rabbits, but reported that initial and ultimate pH depended on severity of death struggle and the level of feeding and degree of fatigue immediately before death. Marsh (1981), however, reported that different animals have different rates of ATP fall and that the completion of rigor takes longer in beef than in the rabbit. The difference in glycolytic changes between red and white fibers was reviewed by Cassens and Cooper (1971). In the review, they reported that red fibers had lower glycolytic metabolism and glycogen content than white fibers. This observation was also reported by Forrest et al. (1975).

Tarrant and Mothersill (1977) investigated glycolysis at several locations in six major hindquarter muscles of intact sides of beef. They found that the time required for pH to fall to 6.0 ranged from 2.2 - 13.6 hrs, varying between muscles and depth in the carcass. This variation was also supported by Bendall (1978) who reported the time it took for pH in four major muscles of cooling carcasses to drop from 7.1 or 7.0 to 6.0 was 8 - 16 hrs. Martin and Fredeen (1974) followed the pattern of pH fall in four different muscles of bull, steer and heifer carcasses and concluded that the pattern was similar for the different muscles over the period of 0 - 10 hr postmortem, but the muscles significantly differed in all pH measurements from 2 - 24 hrs. On the basis of the findings (Martin and Fredeen, 1974), the muscles were classified as fast, medium and slow glycolyzing muscles. Other factors such as addition of sodium chloride (Hamm, 1977; Newbold and Lee, 1965), phosphates (Bernthal, 1987; Hamm, 1977), electrical stimulation (Chrystall and Devine, 1978; Bendall, 1976), temperature (Hamm, 1982; Honikel et al., 1981b; Jolley et al., 1981), comminution (Hamm, 1982; Honikel et al., 1981a) and stress (Lawrie, 1985; Wood and Richards, 1975) were reported to affect postmortem glycolysis in different animals.

Deviation from the normal course of post-mortem changes have been reported to cause variation in meat quality

(Lawrie, 1985; Forrest et al., 1975; Khan and Lenz, 1973). Martin and Fredeen (1974) reported that problems such as pale, soft exudative pork (PSE) and dark cutting beef are specific examples of undesirable changes in muscles. Conditions such as thaw rigor and cold shortening in beef and lamb perhaps are the most conspicuous problems that require knowledge of early postmortem events for their solution (Marsh, 1981; Forrest et al., 1975). Honikel and Fisher (1977) reported that PSE muscles at 45 min postmortem had very low levels of ATP, glycogen and pH but elevated values of IMP and lactate while dark firm and dry (DFD) muscles had low levels of ATP, glycogen and lactate in combination with high pH at the same period of time. DFD muscles were reported to have a final pH of > 6.2 (Fjelkner-Modig and Ruderus, 1983a, b). Polthaust and Hamm (1976) as cited by Hamm (1986) suggested that both PSE and DFD muscles of stress susceptible pigs were caused by accelerated anaerobic glycolysis. However, in DFD muscles glycogen was metabolized in the period preceding death, while in PSE muscles accelerated glycolysis occurs after death. Similarly, Cassens et al. (1963) observed that porcine muscles that went into rigor rapidly at a low pH and high temperature ultimately appeared soft, pale and watery and muscles that went into rigor rapidly at a high pH and reduced temperature appeared dark, firm and dry. An extensive comparison of processing properties of 50 normal and 50 PSE

loins was reported by Merkel (1971). The results indicated heat processing and freezer storage losses were greater in PSE muscles compared to normal one. Also PSE muscles were more tender, higher in thiobarbituric acid (TBA) values and lower in emulsifying capacity than normal muscles.

Tenderness in Meat

The importance of tenderness in meat is underscored by the fact that it has been so extensively studied (Calkins and Seideman, 1988; Koohmaraie et al., 1988a, b; Cross, 1987, Lawrie, 1985; Stanley, 1983; Karmas, 1970). As early as 1947, Deatherage and Harsham had reported that tenderness was an important factor that consumers desired. For this reason, considerable time and money has been spent researching the factors involved in tenderness and how those factors can be manipulated to improve this desirable quality in meat. Scientists have used genetic, nutritional, chemical, microstructural, instrumental and sensory methodology to understand the factors controlling tenderness (Stanley, 1983). McGill (1981), however, observed that consumers place tenderness in different priority levels for different products. Flavor and juiciness are considered more important in pork and lamb whereas tenderness is considered top priority in beef.

Several theories have been advanced in an attempt to explain tenderness-toughness in meat. The most widely accepted theories are those that relate tenderness-toughness to myofibrillar contraction and/or connective tissue (Stanley, 1983; Marsh, 1977). Currie and Wolfe (1980) reported that intrafiber water in beef is a significant contributing factor to the tensile strength and adhesive

properties of muscle. The role of cytoskeleton in meat tenderness was also advanced (Robson and Huiatt, 1983; Stanley, 1983). In spite of the numerous existing theories, tenderness remains an elusive and poorly understood attribute. However, it is widely believed that postmortem conditions and treatment outweigh live animal factors in determining tenderness in meat (Cross, 1979; Locker, 1985). Some of the postmortem conditions and treatments that contribute to toughness or the reduction of it are discussed in the following paragraphs:

Cold Shortening, Thaw Shortening and Cold-induced Toughening

Muscles can enter rigor mortis with different degrees of overlap between the thick and thin filaments (Davey,1984). Severe shortening has been shown to occur when carcasses were exposed to low temperatures early post-mortem (Marsh et al., 1968; Locker and Hagyard, 1963). For muscles of normal pH (5.4 - 5.6) shear force was found to be highly dependent on muscle fiber contraction (Bouton et al., 1973a). In an extensive review of cold shortening it was noted that shortening of muscles by cold shock or thaw rigor or both was a major cause of meat toughness and that either of these factors may outweigh other factors such as age, breed, sex, plane of nutrition, grade or pre-slaughter stress on their effect on tenderness (Locker et al., 1975). Davey (1984) reported that an unrestrained prerigor ox M.

sternomandibularis shortened by up to 50% of its excised length when held at about 2°C for about 24 hrs. The magnitude of contracture increased with falling temperature and was reduced when chilling was delayed. Locker and Hagyard (1963) reported that isolated fresh beef muscles shortened more at 2°C than at 37°C with minimum shortening occurring in the temperature region of 14-18°C. The biochemical basis for cold shortening was reviewed by Locker (1985) in which he reported that cold or thaw shortening was triggered by the release of calcium from the sarcoplasmic reticulum (SR) and mitochondria. Wood and Richards (1974a, b) explained cold shortening in broiler muscles to be as a result of a salt "flux" of Ca^{2+} that is released from the sarcoplasmic reticulum (SR) stimulating myofibrillar ATP to split effecting shortening. The release of Ca^{2+} from SR and mitochondria was also reported by Davey and Gilbert (1974). Cornforth et al.(1980) explained that the reason why white (rabbit) muscle does not cold shorten whereas red muscle (beef) does, is because of the difference in the amount of mitochondria and SR membranes between white and red muscles.

Since establishing the fact that shortening of muscles induced toughening, there have been several investigations that attempted to reduce this effect. Davey and Gilbert (1973, 1974) avoided cold shortening in lambs by placing prerigor carcass in standing position during freezing and then holding the carcass for 20 days in frozen

storage to prevent thaw rigor shortening. Bouton et al. (1973b, 1974) reported that pelvic suspension reduced cold shortening in lamb carcasses. It was demonstrated by Smith et al. (1971) that tenderness in beef was increased by severance of vertebrae and ligamentum nuchae, suspension via obturator foramen and attachments of weights.

In addition to skeletal restraints, other measures such as high temperature conditioning (Henrickson and Asghar, 1985; Dutson et al., 1977; Busch et al., 1967) and electrical stimulation (Chrystall and Devine, 1985, 1978; Cross, 1979) were found to affect muscle shortening and consequently tenderness.

High Temperature Conditioning

High temperature conditioning has been shown to reduce the effect of cold shortening and improve tenderness. Busch et al. (1967) compared differences in post-mortem muscle shortening at 2°C and 37°C. Their results showed that shortening began sooner postmortem at 2°C than at 37°C. More tension was developed at 2°C than at 37°C and at 37°C shortening began at pH < 6.0, while shortening began at pH > 6.0 at 2°C. Smith et al. (1971) found that holding carcasses at 16°C for the initial 16 - 20 hr postmortem yielded greater reduction in shear force values compared to those from carcasses held at chill room temperatures. Bouton et al. (1974) compared lamb muscles at

0-1°C and 7-8°C conditioning temperatures and reported that myofibrillar toughness was reduced at 7-8°C compared to 0-1°C.

The idea that high temperature contributed to tenderness by only preventing cold shortening (Locker and Hagyard, 1963) has been questioned by a number of studies. Cassens et al. (1963) demonstrated that changes in porcine muscle structure during the first 24 hrs post-mortem of elevated temperatures included the disruption of sarcoplasmic components and some disorganization in the myofibrils. Busch et al. (1967) in a study of bovine muscles stored at 37°C suggested other factors (apart from lack of cold shortening) which are temperature dependent as responsible for continued decrease in shear resistance values observed. Lochner et al. (1980) studied the effect of early postmortem cooling rate on beef tenderness and found that, except in very rapidly chilled lean carcasses, cold shortening was not a significant determinant of tenderness. The mechanisms by which temperature conditioning impacts tenderness has been advanced by several authors. Moeller et al. (1976, 1977) reported that tenderness produced at high temperature in bovine muscles studied was due to increased activity of lysosomal enzymes during the first 24 hr post-mortem. Compared to 2°C, incubation of muscles at 37°C was found to increase the release of lysosomal enzymes (Wu et al., 1981). Dutson (1983a) reported that postmortem

temperature and pH had effect on meat tenderness and disruption of specific myofibrillar proteins. He (Dutson, 1983a) observed that while degradation of troponin T, Z-lines, gap filaments and connectin was increased at both high and low pH at 37°C, degradation of myosin was only increased at low pH at that temperature. Therefore, it was the conclusion of these authors (Dutson, 1983a; Dutson et al., 1982, 1977; Wu et al., 1981; Moeller et al., 1977, 1976) that the enzyme systems most responsible for degradation of myofibrillar protein were more active at high (37°C) temperature and lower pH. Contrary to this, however, is the report by Marsh (1981) and Marsh et al. (1981) that tenderization of steaks from beef was due primarily to enzymes or enzyme systems that were active at near neutral pH and a temperature of about 3°C. It was the conclusion of Marsh (1983) that tenderness proceeds faster in meat when pH is higher than normal.

Electrical Stimulation

Electrical stimulation of pre-rigor muscles has received considerable attention as a means of increasing tenderness or alleviating the effect of cold shortening (Cross, 1979). Chrystall and Devine (1985) stated that inducing early rigor through carcass electrical stimulation reduced the risk of toughening that is caused by cold shortening during chilling and thaw shortening during

thawing. Bendall et al. (1976) reported that electrical stimulation induced a pH fall to 6.0 and 5.7 within 1 hr and 2-1/2 hrs, respectively, in the major muscles of the forelimb, back and thigh of beef carcasses. According to Bendall et al. (1976), this change represented a gain of more than 8 hrs over the time required for unstimulated carcasses held at 16°C to reach that temperature. Electrical stimulation has been shown to improve tenderness, induce rapid pH fall and temperature increase and to significantly lower pH values and improve protein extractability compared to unstimulated muscles (Samejima et al., 1986; Wu et al., 1985; Rashid et al., 1983b; Elgasim et al., 1981; McKeith et al., 1980; Bouton et al., 1978; Savell et al., 1978). Chrystall and Devine (1978) reported that the time to apply electrical stimulation for maximum achievable effect is when the muscle temperature is still high. Electrical stimulation parameters of high voltage and low frequency were found to be more effective in accelerating postmortem glycolysis in lambs compared to low voltage with high frequency (Rashid et al., 1983a). The mechanism involved in the improvement of tenderness by electrical stimulation was discussed in several studies. Whiting et al. (1981) indicated electrical stimulation increased sarcomere length of lamb muscles but did not have any effect on water-holding capacity, protein solubility and emulsifying capacity. Contrary to the finding of Whiting et al. (1981) on sarcomere length, Elgasim et al.

(1981) and Savell et al. (1978) did not find any significant difference in sarcomere length between stimulated and non-stimulated beef carcasses. Their observation led to the suggestion that some mechanism other than, or in addition to, prevention of cold shortening was responsible for the improvement in meat tenderness with electrical stimulation. Structural damage due to formation of contraction band was suggested as the reason for electrical stimulation improvement of tenderness (McKeith et al., 1980; Savell et al., 1978). These studies were supported by Sorinmade et al. (1982) when they showed that electrically stimulated beef muscles had irregular contraction bands, superstretched myofibrils, absence or ill defined I bands, A bands or Z-lines, and torn and fragmented myofibrils at the Z-line. Higher myofibrillar fragmentation index and increased autolytic process was reported for electrically stimulated muscles (Fabianson and Libelius, 1985; Sonaiya et al., 1982; Sorinmade et al.,1982).

Postmortem Proteolysis and Some Enzymes Involved

Aging of carcasses for 8 - 14 days at 0° - 4°C to improve tenderness has been practiced for many years and still remains an important procedure for producing tender meat (Koohmaraie et al., 1986). The proteolysis of myofibrillar protein by some endogenous proteolytic enzymes are reported to be a key event in tenderization during postmortem storage of meat (Asghar and Bhatti, 1987; Goll et al., 1983; Davey and Gilbert, 1983). Asghar and Bhatti (1987) stated that under identical conditions, post-mortem tenderization in carcass of different species falls in the order: avian > porcine > ovine > bovine.

In a study of protein changes during postmortem tenderization of poultry meat, Khan and Van Den Berg (1964) found that the buffer-extractable nitrogen decreased rapidly after death during the onset of rigor and gradually increased to maximum value during postrigor aging. The authors (Khan and Van Den Berg, 1964) explained the changes in extractable nitrogen as resulting from changes in solubility of myofibrillar protein and concluded that proteolysis weakened or broke the bonds that bound myofibrils to the matrix of the muscles which lead to postrigor tenderization. Hegarty et al. (1963a) observed that protein solubility was directly related to meat tenderness during postmortem storage of beef. Goll et al. (1964) found that sarcoplasmic protein solubility was highest in beef

immediately after slaughter but concluded that there is no relationship between protein solubility and tenderness. The lack of relationship between protein solubility and tenderness was also reported by Chaudhry et al. (1969). Parish et al. (1969) studied the relationship between proteolysis and tenderness and reported that nonprotein nitrogen (NPN) and free amino acids increased during postmortem aging indicating degradation of protein and/or peptides. The authors concluded that tenderization was caused by the weakening of Z-disks and thin filaments. Other changes in myofibrillar proteins during aging includes degradation of myosin heavy chain, gradual degradation of troponin-T and the appearance of a 30,000 dalton component (Bechtel and Parish, 1983; Yamamoto et al., 1979; Cheng and Parrish, 1978). These changes were reported to be highly dependent on temperature (Bechtel and Parish, 1983).

Enzymes which have the ability to hydrolyze peptide bonds are referred to as proteinases (Asghar and Bhatti, 1987). These enzymes are generally believed to be responsible for some of the changes that take place during post-mortem aging of meat. However, the role of various proteinases in muscle proteolysis is still a subject of controversy. Some researchers (Dutson, 1983a; Moeller et al., 1976, 1977) consider lysosomal proteinases to be responsible for post-mortem proteolysis while others (Koohmaraie et al., 1988a, b; Dayton et al., 1976) consider nonlysosomal

proteinases as responsible. Yet others (Calkins and Seideman, 1988; Goll et al., 1983) believe that combination of lysosomal and nonlysosomal calcium-dependent proteases (CDPs) are responsible for post-mortem tenderization. CDPs have been reported to be synonymous with calcium-activated neutral protease (CANP), neutral calcium-activated protease (NCAP), kinase activating factor (KAF), calcium-activated factor (CAF), calcium-activated sarcoplasmic factor (CASF) and calpain (Asghar and Bhatti, 1987). The existence of two forms: high Ca^{2+}-requiring CDP (CDP-II) and low Ca^{2+}-requiring CDP (CDP-I) was reported by Koohmaraie et al. (1987). Goll et al. (1983) studied the proteolytic enzymes that are likely to be important causative agents in postmortem meat tenderization. Their findings showed that only 7 of the 13 known lysosomal enzymes (Cathepsins A, B, C, D, H and L and carboxypeptidase B) were located inside skeletal muscle cells and only one other protease (CAF) was located in muscle cells. This study (Goll et al., 1983) concluded that CAF was responsible for most (70%) proteolytic changes at 0° - 4°C, whereas cathepsins L > H > B in conjunction with CAF acted under frozen or 16°C storage temperature. Calkins and Seideman (1988) compared CDP-1 and Cathepsin B and H in terms of relative importance to meat tenderness and the changes associated with post-mortem aging. They found that CDP-1 helped to establish initial (day 1) meat tenderness, but cathepsin B and H were responsible for the tenderization that

occurred during aging. An earlier study (Busch et al., 1972) demonstrated that incubation of rabbit psoas strips in saline solution containing 1 mM Ca^{2+} and 5 mM Mg^{2+} caused complete Z-line removal, and the authors concluded that an endogenous protein that catalyzed the degradation of myofibril was regulated by calcium. Koohmaraie et al. (1988a, b) infused lamb and bovine muscles with calcium chloride and studied the treatment effect on postmortem tenderization process. They concluded that calcium dependent protease (CDP) was responsible for the observed postmortem proteolysis and tenderization with no effect of cathepsins B, H and L observed.

A number of other studies (Koohmaraie et al., 1990, 1987, 1986, 1984b; Suzuki et al., 1982; Penny et al., 1974) ascertained the importance of CDP in post-mortem tenderization. The effect of CAF on bovine myofibrillar proteins included solubilization of myofibrillar proteins, degradation of titin and increased myofibrillar fragmentation (Zeece et al., 1986). CAF was also reported to hydrolyze tropomyosin, troponins I, T and the M and C proteins of myofibrils. The hydrolysis of troponin T produces a 30,000 dalton protein which can be used as an indicator of meat tenderness (Davey, 1983). Contrary to the studies which indicated CAF had effect on tenderness, Takahashi et al. (1987), Rodemann et al. (1982), Hattori et al. (1979) did not find CAF important in inducing postmortem tenderization.

Postmortem Changes and Their Effect on Some Functional Properties of Meat

a - Extractable Protein (EP)

When meat is minced for the production of emulsion type sausages, the muscle cell membrane (sarcolemma) is destroyed, the constituent myofilaments of myofibrils are no longer restrained and the myofibrillar system swells. At the surface of contact between the swollen particles and surrounding fluids (sarcoplasm and added water) varying amounts of myofibrillar proteins are dissolved (Hamm, 1986). The component of the myofibrills mainly responsible for the swelling and water binding are the proteins myosin and actin. These two proteins represent about 65% of the myofibrillar protein and about 50% of the total muscle protein (Greaser et al., 1989).

Muscle proteins are generally divided into 3 broad categories: 1) The salt-soluble (myofibrillar) proteins defined as those proteins which are soluble in salt solution and insoluble in pure water, 2) Water-soluble (sarcoplasmic) proteins which are soluble in water or dilute salt solution and 3) Those proteins (stroma) that do not dissolve in either water or salt solution at low temperatures (Lawrie, 1985; Forrest et al., 1975; Saffle, 1968). The salt soluble proteins (actin, myosin, actomyosin) were reported to be more effective than the water soluble proteins (myoglobin, hemoglobin, enzymes associated with glycolysis and citric acid cycle) in preparing emulsions

(Trautman, 1964, 1966; Fukazawa et al., 1961a, b; Swift et al., 1961). Hegarty et al. (1963a, b) compared the emulsifying capacity of beef muscle actin, myosin, actomyosin and sarcoplasmic proteins and found that myosin and actomyosin produced emulsions with superior stability at or near neutral pH. At pH of normal fresh meat (5.6 - 5.8) the sarcoplasmic fractions produced the most stable emulsions, while actin was found to produce very stable emulsion under all conditions.

Several factors were reported to affect the protein extractability of muscle proteins. The solubilization of sarcoplasmic and myofibrillar protein was studied in porcine muscles at the time of slaughter, at the onset of rigor mortis, upon completion of rigor and 24 h postmortem (Sayre and Briskey, 1963). Their results indicated that sarcoplasmic protein solubility decreased at 24 hrs to 55% and 17% of that found at 0 hr in muscles exhibiting high temperature and low pH and high temperature and high pH at onset of rigor, respectively. Furthermore, myofibrillar protein solubility decreased by about 75% in muscles exhibiting high temperature and low pH at onset of rigor (Sayre and Briskey, 1963). Trautman (1966) reported that initial prerigor pH may determine subsequent characteristics of salt soluble proteins in ham muscles. Davey and Gilbert (1968), however, reported that the rate and extent of protein extractability depended on the ultimate pH of beef

and rabbit carcasses. Salt-soluble proteins of various meats were studied by Saffle and Galbreath (1964). Their result demonstrated that pH of meat had significant effects on the amount of salt-soluble proteins which could be extracted. As pH increased, the amount of protein which could be extracted also increased. Kolczak and Weber (1969) performed a study on water solubility of porcine muscle (M. longissimus) protein and found that the solubility of sarcoplasmic protein depended on the quality of muscle and was lowest in pale, soft exudative muscles. Low pH and high temperature was advanced as the reason for the reduced extractability of sarcoplasmic proteins in PSE muscles (Kolczak and Weber, 1969). Merkel (1971) showed that PSE muscles had lower emulsifying capacity than normal muscles. In general, pre-rigor muscles have higher percent extractable proteins than post-rigor muscles (Bernthal et al., 1989; Honikel et al., 1981a; Saffle and Galbreath, 1964; Trautman, 1964). It was explained by Hamm (1981) and Honikel et al. (1981a) that the decrease in protein solubility during prerigor phase is caused by the fall in pH, but loss of solubility below pH 6.1 is caused by development of rigor mortis. However, in an earlier study, Landes et al. (1971) reported that total extractable nitrogen, total soluble fibrillar protein nitrogen and soluble actomyosin nitrogen of turkey breast muscle continued to increase during the early post-mortem period.

The effects of temperature and time on protein solubility of rabbit and bovine muscles were investigated (Chaudhry 1969). The results showed that myofibrillar protein solubility in salt solution increased markedly with increased postmortem storage temperature up to 25°C. Acton (1972) did not find any significant changes in the quantity of protein extracted during cooking of poultry meat loaves between 4-35°C, but percent extractable protein was significantly reduced at higher internal temperatures. The influence of added phosphates on the extractability of denatured and native myofibrils in 0.6 N NaCl was investigated by Fukazawa et al. (1961c). They found that the effect of phosphate on amount of extracted protein in native myofibrils was in the order: pyrophosphate (PP) > tripolyphosphate (TPP) > hexametaphosphate (HMP), and only PP had any effect on denatured fibrils. Bernthal (1987) indicated that addition of tetrasodium polyphosphate (TSPP) to pre-rigor and post-rigor beef muscle homogenates increased protein extractability in a 1.0 N NaCl solution. Bernthal et al. (1989) demonstrated that NaCl increased protein extractability in pre-rigor and post-rigor ground beef. Paterson et al. (1988) reported that NaCl (1.0 M > 0.7 M > 0.4M) increased the extraction of titin, myosin and other myofibrillar proteins from beef. This effect was enhanced when 10 mM PP was added to NaCl solutions.

b - Water-holding Capacity (WHC)

Fresh lean meat at slaughter contains about 75% water. Eighty-five to ninety-five percent of this water is held within the fiber in dynamic equilibrium with the remaining 5-15% plasma fluid outside the myofiber walls. This amount of water (75%) is subsequently subjected to considerable variation due to the gains that occur during processing or the losses that occurs through drip, cooking or evaporation. The retention of the water in meat and that of any added water during storage and further processing is of great importance to meat industry (Paterson et al., 1988; Offer and Trinick, 1983; Penny, 1975). According to Forrest et al. (1975) and Hamm (1960), the ability of meat to hold on to its water or added water during application of force is termed water-holding capacity. Water-holding capacity is a very important feature of meat quality because of its close relation to taste, tenderness, color, juiciness, firmness and other quality factors (Paterson et al., 1988; Lawrie, 1985; Offer and Trinick, 1983; Wismer-Pedersen, 1978; Forrest et al., 1975; Hamm, 1960). According to Forrest et al. (1975), water in meat exists in bound, immobilized and free form. About 4% of muscle water exists in bound form being bound to the hydrophilic groups on proteins (Lawrie, 1985; Forrest et al., 1975; Hamm, 1960). Hamm (1960) reported that the hydrophilic groups that are responsible for the strong binding

of water involved polar groups of the side chains of protein, amino, carboxyl and imido groups of peptide bonds as well as hydrogen bonding. The immobilized water is attracted to the bound water in layers for which bonding forces are progressively weakened with distance from the bound water (Forrest et al., 1975). Offer and Trinick (1983) hypothesized that gains or losses of water in meat is due to the swelling or shrinking of the myofibrills by expansion or shrinking of filament lattice. Several factors have been shown to affect water-holding capacity of meat. Among other factors, pH, metals, species of animals, sex, age, grade, breeding conditions and treatments of animals before and after slaughter and postmortem changes affect water-holding capacity of meat (Hamm, 1960). Kauffman et al. (1986) indicated that water-holding capacity is enhanced at pH values considerably above or below the isoelectric point of muscle. Considerable variation exists in the water-holding capacity of meat from different species (Wismer-Pedersen, 1978). On average, the variation in pork is greater than that in beef; while variation in beef is equal to that of horse and greater than that of poultry meat. Kauffman (1986) measured the relative waterholding capacity of muscles by measuring the amount of drip loss during centrifugation. Drip loss in intact M. semitendinosus, M. adductor, M. semimembranosus and M. bicep femoris varied among the muscles and with the depth at which muscle sample

was obtained. Studies on pork by Taylor (1972) showed that breeds differed significantly ($P<0.05$) in susceptibility to drip loss which was explained by differences in the rate of postmortem pH decline. Honikel et al. (1981a, b) investigated changes in water-holding capacity of bovine neck muscles at various temperatures by measuring cooking loss of unsalted and salted (2% NaCl) muscle homogenates. They (Honikel et al., 1981a, b) found that with a decrease of pH of intact tissue, water-holding capacity of salted homogenates decreased slowly post-mortem until pH 6.2, and a large decrease in water-holding capacity occurred during development of rigor mortis. However, very slight changes took place in water-holding capacity of unsalted muscle homogenates with decrease in pH. It was the conclusion of the authors (Honikel et al., 1981a, b) that only one-third of the decrease in water-holding capacity was due to pH and the rest was due to rigor mortis itself. Using the filter paper method of measuring water-holding capacity, Jolley et al. (1981) showed that water-holding capacity of raw intact bovine neck muscles was not influenced by development of rigor. Other factors such as the addition of salt was found to improve water-holding capacity in different meats (Paterson et al., 1988; Zapata and Price, 1982; Honikel et al., 1981a, b; Wiebicki et al., 1957). The effect of adding citric acid and the chlorides of sodium, potassium, calcium and magnesium on water-holding was

investigated by Wiebicki et al. (1957). Addition of sodium, potassium, calcium and magnesium chlorides to meat prior to heating increased water-holding capacity of meat protein when heated to 70°C. Citric acid increased shrinkage when added to meat prior to heating.

Other factors such as rate of freezing (Anon and Calvelo, 1980; Khan and Lenz, 1972; Bailey, 1972), rate of thawing (James et al., 1984), length of freezer storage (Jeremiah et al., 1981a, b and 1982; Khan and Lenz, 1977), anatomical distribution or muscle type (Taylor, 1972) impact upon water-holding capacity of meat. Methods of measuring water-holding capacity in meat and their usefulness have been objects of several studies (Bernthal et al., 1989; Kauffman et al., 1986; Wismer-Pedersen, 1978; Penny, 1975; Hamm, 1960).

c - Color

Color, according to MacDougall (1982), is a subjective experience. It is a result of a combination of several factors. Any specific color has three attributes known as hue, chroma and value (Forrest et al., 1975). Color of meat is probably the single most important factor that affects the marketability of fresh retail meat cuts (Kropf, 1980; Westerberg, 1971). As stated by Seideman et al. (1984), color of fresh meat is important to every aspect of the meat industry. The discoloration of fresh beef in

pre-packaged consumer cuts was related to the limit of shelf life and delayed the adoption of centralized beef cutting and packaging operations (Hood, 1975).

The pigment responsible for the color of meat is the heme protein, myoglobin, along with the residual quantities of hemoglobin (MacDougal, 1982). The color of meat is partially dependent on the oxidation state of iron within the heme ring of the color pigment of meat (Seideman et al., 1984; Ramsbottom, 1978; Forrest et al., 1975). According to Seideman et al. (1984) and Huffman (1980), myoglobin can exist in three forms: purple reduced anoxic myoglobin, red reduced oxymyoglobin and brown metmyoglobin (oxidized). Bertelsen and Skibsted (1987) and Rizvi (1981) stated that the color of fresh and frozen meat is largely determined by the proportional amounts of deoxymyoglobin, oxymyoglobin and metmyoglobin. It was observed by Ledward et al. (1986) and Ledward (1971) that in the absence of evaporation, loss of quality during retail display is due almost exclusively to the formation of metmyoglobin. In a study of consumer acceptance of pre-packaged meat on display, Hood and Riordan (1973) found shoppers discriminated against those samples with higher metmyoglobin content.

The factors affecting the color of meat have been extensively studied. Seideman et al. (1984) reported that color intensity of meat is determined by such antemortem factors as species, stress, sex, age, post-mortem pH and

rate of decline and ultimate pH of the meat. Furthermore, Seideman et al. (1984) reported that color difference between species is due to differing concentration of myoglobin. Beef has a higher concentration of myoglobin, therefore, it is darker than lamb or pork. Also, male animals usually produce darker meat than females due to greater concentration of color pigments. According to Satterlee and Hansmeyer (1974), the stability of pigment in intact meat involves such factors as oxygen penetration, microbial growth, fat oxidation, presence of flavin compounds and oxygen permeability of packaging films. Walker (1980) reported that bacterial growth can contribute significantly to color deterioration of fresh meat due to reduction in oxygen concentration, changes in pH, production of proteolytic enzymes and changes in relative humidity.

In a study by Hood (1980), 45% and 32% of variations in muscle color in beef was found to be due to differences in muscle and temperature, respectively. The color stability of muscles as affected by storage temperature at 0, 5 and 10°C was reported to be in the order: M. psoas major < M. gluteus medius < M. semimembranosus < M. longissimus which was the most stable with respect to color (Hood, 1980). This observation is in agreement with an earlier (Hood, 1975) study which indicated that M. psoas major and M. gluteus medius were less stable than M. semimembranosus and M. longissimus in terms of color. The anatomical location of

muscle was reported to affect the susceptibility of muscle to metmyoglobin formation. The susceptability of four beef muscles to metmyoglobin formation was in the order: beef biceps femoris > semimembranosus > longissimus > semitendinosus muscles (Ledward, 1971). According to Ledward (1985), the temperature at which muscles go into rigor and the time the muscles are held at that temperature modified the subsequent rate of metmyoglobin formation. Ledward (1985) and Hood (1980) reported that the rate of metmyoglobin accumulation in beef increased with increased storage temperature. Although no effect of pH could be found on discoloration in beef, Hood, (1980), Ledward (1985), and Nichols and Cross (1980) observed that severe nonuniformity in terms of color in muscles at the deeper part of carcasses was due to combined activity of high temperature and low pH found in those muscles. High temperatures and low pH could affect the reducing activity of mitochondrial enzymes responsible for the rate at which metmyoglobin accumulate on surface of beef muscle (Ledward, 1985).

In a study by Ledward et al. (1986) and Nichols and Cross (1980), electrical stimulation was found to have no significant effect on the color stability of semimembranosus and longissimus muscles of beef. Other factors such as humidity (Ledward, 1971), excision time (Nichols and Cross, 1980), sodium ascorbate (Hood, 1975), amount of light

(Hood, 1980) and packaging film (Hood, 1980, Ledward, 1971) were reported to affect the color of meat.

Methods of measuring meat color are numerous and varied. They all, however, fall into two basic categories: visual appraisal and instrumental analysis (Hunt, 1980). Farouk et al. (1990), Ledward (1986) and Eikelenboom (1985) used a Hunter lab color difference meter to determine the color of meat.

Tenderization of Meat by Infusion

Tenderization of tough meat has been a problem since ancient times, the importance of which is shown by the large number of processes which have been developed in an attempt to induce tenderness in meat (Karmas, 1970). Sears (1989) described a cardiovascular infusion process for whole beef carcass tenderization (of slaughtered animals immediately after bleeding) using a pumping unit that displaces the remaining blood with glucose solution. Karmas (1970) reported significant tenderization when water was injected into carcasses before completion of rigor. He explained that the water when injected under pressure will spread muscle fibers and get distributed uniformly throughout the meat. After completion of rigor, the water is then set and held as true hydration water. Increased tenderness is possibly due to increased moisture content. Injection of freshly slaughtered carcass with 1-3% by weight of water at pressure of 40-600 psi and at body temperature was reported to improve tenderness by about 25% (William, 1961, U.S. patent #3006.768, cited by Karmas, 1970). The rationale for improved tenderness was that water injection activates the natural enzymes in meat. Also, the use of water at body temperature slows the rate of chill of meat and therefore stimulates rigor mortis; and the quicker

rigor mortis sets in, the quicker tenderization is accomplished.

Koohmaraie et al. (1988b, 1989) reported a procedure which will eliminate the requirement for post-mortem storage beyond 24 h to ensure meat tenderness. The procedure involved low frequency electrical stimulation of ovine carcasses after death followed by infusion with a solution containing calcium chloride prior to evisceration. Results of the studies showed that this method significantly reduced shear force values measured at 24 h postmortem. The studies, however, did not find any increased tenderness due to infusion of carcasses with water alone.

Additives such as gelatin, phosphates, salts of isotonic or hypotonic proportions may be incorporated in infusion solutions for the purpose of improving tenderness (William 1961, 1964, U.S. patents #3.006.768 and #3.49.696 as cited by Karmas, 1970). Some of the additives used in tenderizing solutions are discussed in the following paragraphs:

a - Glycerine or Glycerol

Literature on the importance of this additive to tenderness is scanty. However, glycerol is nontoxic, nonvolatile and completely miscible with water. Glycerin is also highly hygroscopic and because of this quality, it is used to retain moisture, prevent evaporation and increase shelf life in foods. It has a stabilizing and color

enhancing effect and reduces protein denaturation (Pendleton, 1970, Anon, 1968). Karmas (1970) presented a list of ingredients which included glycerin as useful in tenderizing beef but did not exactly specify what role glycerine played in the mixture. It was reported by Whiting that glycerol significantly reduced the amount of water exudation from meat batters.

In a study by Lacroix and Castaigne (1985), the effect of added glycerol on texture of frankfurter type meat emulsion was investigated. It was found that glycerol reduced water loss during cooking due to its interaction with the meat proteins. The authors (Lacroix and Castaigne, 1985) postulated that glycerol might act as a potential substitute for the water used in protein hydration and as a protective agent during thermal degradation process. However, a loss of eating quality was reported in glycerol treated beef (Webster et al., 1982; Morgan and Farkas, 1978). Therefore, the effect of glycerine on tenderness might be due to increased water-holding capacity or due to its effect (when present in interfilament medium) on the interfilament distance of muscle fibers (Rome, 1967, 1968, 1972).

b - <u>Dextrose and Maltose (Sugars)</u>

Schleich and Arnold (1962) as cited by Karmas (1970) patented a tenderizing process that involved stitch pumping or

pumping through the arterial system a solution containing 8.5 parts by weight of dextrose and 2.8 parts by weight of sucrose. Rees (1988) reported a tenderizing process that employed sugar to tenderize lower grades of meat. The addition of sugars and complex carbohydrates was reported to increase water binding, stabilize hydrophobic interaction and increase thermal stability of globular proteins (Whiting, 1987). Newbold and Lee (1965) demonstrated that added glycolizable substrates like glucose to sheep, rabbit and ox muscle minces resulted in marked lowering of ultimate pH. Therefore, the effect of adding sugars to improve tenderness could be to rapidly lower pH while temperature of muscle is still high. Dutson et al. (1982), Wu et al. (1981) and Moeller et al. (1976, 1977) suggested that higher temperature and low pH were necessary for improved meat tenderness.

c - Calcium chloride ($CaCl_2$)

Since it was suggested that an enzyme existed that required calcium to catalyze the degradation of myofibril (Busch et al., 1972), many studies were reported that proved calcium dependent proteases are activated by Ca^{2+} (Koohmaraie et al., 1988a, b; 1989; Zeece et al., 1986; Penny et al., 1974). However, others (Takahashi et al., 1987) argued that ions (Ca^{2+}) are directly responsible for the degradation of

myofibril and not through the activation of CDPs.

Wood and Richards (1974a) reported that addition of 1 mM Ca^{2+} significantly ($P<0.001$) lowered the amount of tension developed in broiler chicken. Bovine muscles excised after slaughter were treated (Koohmaraie et al., 1988a) with calcium chloride, and post-mortem changes were found to be completed in 24 h. In another study (Koohmaraie et al., 1988b), lamb carcasses were infused with .3 M $CaCl_2$ immediately after death. Tenderness was accelerated and proteolysis of myofibrillar protein was completed within 24 h. The authors (Koohmaraie et al., 1988b) suggested that activation of CDPs was responsible for the acceleration of these events. Koohmaraie et al. (1989) arterially infused ovine carcasses immediately after slaughter and electrical stimulation with 10% by weight (live) solution containing 0.075 M, 0.15 M and 0.3 M $CaCl_2$. The result obtained indicated that 0.3 M $CaCl_2$ concentration was the most effective in reducing shear force after 24 h postmortem. In order to determine whether increased tenderness was due to increased ionic strength, the authors (Koohmaraie et al., 1989) infused some of the carcasses with a solution of sodium chloride. Infused carcasses were found to be more tender after 6 days of post-mortem storage, but not after one day as was observed with $CaCl_2$ infused carcasses. Therefore, they concluded that increased tenderness was not

due to increased ionic strength. Like the previous studies, activation of CDP by $CaCl_2$ was given as the cause for observed tenderness.

d - Phosphates

Phosphates are important functional additives with diverse use in variety of foods including meat, poultry and seafood (Sofos, 1986). Specific benefits of using polyphosphates in various red meats, poultry and seafood include improved stability of cured meat color, reduced cook out juicies in canned hams, reduced fluid losses during cooking, greater juiciness, greater tenderness, improved flavor, reduced cook time, reduced refrigeration weep, increased protein extraction, stable cooked meat flavor, color and odor, and reduced thawing drip (Sofos, 1986; Pearson and Tauber, 1984; Offer and Trinick, 1983). Hamm (1977) reported that addition of diphosphate to pre-rigor ground beef resulted in acceleration of ATP and glycogen breakdown. Addition of tetrasodium pyrophosphate (TSPP) to pre- and post-rigor beef muscle homogenates resulted in lower ultimate pH compared to homogenates with no TSPP (Bernthal, 1987). Phosphates were also reported to increase water-holding capacity and solubilization of actomyosin (Lewis et al., 1986; Pearson and Tauber, 1984). It was demonstrated by Merkel (1971) that bologna made from PSE and normal porcine muscles containing phosphates were

significantly more tender than those made without phosphates. Fresh meat, including meat from animals such as beef, pork, lamb and mutton, are improved tremendously in tenderness by applying a small amount (4 ounces/100 lb meat) of nontoxic physiologically acceptable basic pyrophosphate salts to meat. Furthermore, dipping, pumping or spraying beef, lamb, pork, chicken or fowl with aqueous solution containing alkali metal pyrophosphate or polyphosphate other than nonlinear phosphates with a small amount of proteolytic enzymes could bring about a substantial amount of tenderization (Delaney 1966; Komarik, 1964, U.S. patents #3.188.213 and #3.147.123, respectively, as cited by Karmas, 1970).

CHAPTER 1

EFFECT OF POST-EXSANGUINATION INFUSION ON THE COMPOSITION, WATER-HOLDING CAPACITY (WHC) AND COLOR OF LAMB

ABSTRACT

Eight lambs each were randomly assigned to one of three treatment groups. One group was infused immediately after bleeding with 10% of live body weight of a solution composed of dextrose (.23%), glycerin (.21%), a phosphate blend (.14%), and maltose (.1%) in tap water (NCa). A second group was infused (10% by weight) with a solution of the same ingredients as above plus .17% $CaCl_2$ (WCa). Another group of eight received no infusion and served as controls (Ctr). Results indicated that in infused carcasses, the amount of retained solution measured as MFF was in the order: shoulder > loin > leg. Within 95% confidence interval, the difference between controls and infused carcasses in MFF never exceeded 2.5%. Infusion had no effects ($P > .05$) on WHC measured as drip and cooking losses in refrigerated samples. However, samples frozen then thawed from infused carcasses have greater thaw drip ($P < .01$) and cooking ($P < .1$) losses than control samples. The amount of drip and cooking losses was in the order: WCa > NCa > Ctr. Frozen storage preserved ($P > .05$) the red color but lowered ($P < .01$) the lightness and

yellowness of ovine muscles (Hunter L, a and b values). The opposite effect was observed following refrigerated storage. Infused samples were lighter ($P < .01$) than control in fresh and frozen samples. They were also yellower ($P < .01$). WCa had less red color ($P < .01$) compared to NCa and Ctr at all times and storage conditions. In freshly cut samples, red color was in the order: NCa > Ctr > WCa. Infusion, particularly with $CaCl_2$, was shown to be detrimental to WHC and color of meat.

INTRODUCTION

Several processes that use the infusion of different ingredients for the purpose of improving tenderness in meat have been patented (Karmas, 1970). Recently interest in improving tenderness of various meats through the infusion process has been renewed (Koohmaraie et al., 1988b, 1990; Sears, 1989; St. Angelo et al., 1991). As noted in chapter 2, infusion of dextrose, maltose, glycerin and polyphosphates significantly improved tenderness in 45 cows compared to the same number of control animals.

If such processes are acceptable by the meat industries, it would interest them to know the effect of the processes on other important qualities of meat. The characteristic of color is very important in the marketability of meat (Westerberg, 1971; Kropf, 1980). The relationship of water-holding capacity (WHC) to meat quality is fully recognized (Wismer-Pedersen, 1987; Paterson et al., 1988; Judge et al., 1989). Similarity exists between the factors that affect these two properties of meat (Seideman et al., 1984; Ledward, 1985; Hamm, 1986).

From the regulatory point of view, the amount of retained solution will determine the acceptability of the process and the labeling of finished products. USDA (1979) regulations stipulate that in the process of tenderization of meat, moisture pickup in treated products must not exceed that of

the untreated products by more than 3%. Farouk et al. (1990) cautioned that in the retail marketing of meat, any attempt to induce certain desirable characteristics to the meat, must avoid causing other problems that would offset an advantage gained. Previously reported studies did not address the above questions of the effect of infusion on composition, color and WHC of infused carcasses; and it is for these reasons that this study was conducted.

MATERIALS AND METHODS

Animals - For this experiment, a total of 24 lambs (6 to 8 mo old, 40 to 52 kg live weight) in groups of three were used; one for each of the three treatments: 1) control (lambs slaughtered and dressed according to MSU Meat Laboratory procedures); 2) infused with 10% of live weight with .1% maltose, .21% glycerin, .23% dextrose, .14% blend of sodium and potassium tripolyphosphate and the remainder water; 3) same as treatment 2 including .17% (.015M) $CaCl_2$. These concentrations were based on preliminary trials conducted.

Infusion and Tissue Sampling - After the lambs were stunned, they were bled and then laid on their back in a pelting cradle. One branch of the carotid artery was exposed and clamped. The other branch was partially severed with a sanitized knife. A sanitized nozzle of a brine pump (Koch No. 011227. Kansas City, MO.) was inserted and secured. Solution was pumped (3.2 kg/cm^2) into the artery until 10% by live weight of the animal was dispensed. After the completion of infusion, carcasses were dressed and transferred to a holding cooler (2°C). Twenty-four hr after slaughter, representative samples of shoulder, leg and loin muscles were taken for proximate analysis. Leg center steaks (2.2 cm thick, 431 g) were used for color and WHC (drip, thaw drip and cooking losses) measurements. The leg steaks were used because they retained the least amount of solution, such that any effect of

infusion shown is likely to be even higher in the other muscles.

Proximate Analysis - Percent moisture, fat and protein were measured as described in the AOAC (1990) methods. The purpose was to determine the distribution of solution in the carcass. The result obtained was used to calculate the percent moisture on fat-free basis (MFF) to adjust for differences in fat content. MFF was calculated as:

% MFF = [{Moisture %/(100-fat%)] x 100

Drip Losses - A total of 48 lamb leg steaks were weighed. Individual steaks were tray-packed (25 type polystyrene foam) with poly-vinylchloride film (PVC, H1-Y-G5 Filmco, Aurora, OH.) having O_2 permeability of 800-1000 mL/24 h. The steaks were then stored 1 wk under simulated retail conditions at 4-5°C and 24 h of fluorescent light (cool white, 90 to 120 ft-c) 38 cm from the packaged steaks. After storage periods, steaks were removed and blotted with tissue paper and weighed. The difference in weight between day zero (d0) and day 7 (d7) was used to calculate percent drip loss.

Thaw Drip Losses - The same number of steaks prepared the same way as described for drip losses were weighed initially before packaging with similar material as was used for drip loss. The steaks were frozen and stored in the dark at -25°C for 1 wk. The frozen steaks were thawed overnight at 4°C

before they were removed from the package, blotted and weighed. Percent thaw drip was estimated as for drip losses.

Cooking Losses - The same steaks used in measuring drip and thaw drip losses were used in measuring cooking losses after the storage period of 1 wk. Steaks were cooked in a Honeywell Convection Oven (Market Forge Co., Everett, MA. Model 1860.2) at an oven temperature of 149°C to an internal temperature of 71°C (the recommended temperature for medium done lamb leg steaks). Amount of cooking losses was calculated as the difference in weight between raw and cooked steaks converted to percentage.

Color - Hunter L, a and b values of steaks previously described under drip and thaw drip losses were measured using Hunter Color Difference Meter (Hunter Lab D25L Optical Sensor. Hunter Associates Laboratory, Inc., Reston, VA.) standardized with pink (L = 66.4, a = 14.4 and b = 8.8) and white (L = 92.2, a = -.9 and b = .1) tiles. Measurements were made through the packaging film. Three readings were taken from each steak and averaged.

Statistical Analysis - The design used for assigning samples for proximate analysis (PA) and steaks for drip, thaw drip and cooking losses (DTC) was a completely randomized design (CRD). Data from these measurements were analyzed by one-way ANOVA. Non-orthogonal contrasts were determined for: Ctr vs NCa and Ctr vs WCa for PA, Ctr vs NCa, Ctr vs WCa and NCa vs WCa for DTC. Treatment means were compared using

Dunnett's and Bonferoni t-tests for PA and DTC, respectively. The design employed for color measurement was a double split plot with repeated measures (Gill, 1978a). The F-ratios for interactions were determined with a Systat computer statistical package (Wilkinson, 1986). The nature of specific comparisons (non-orthogonal) were dictated by the significance of interaction in the double split plot ANOVA. Contrasts were determined for: Ctr vs NCa, Ctr vs WCa and NCa vs WCa. Special error measures were taken from the split plot analysis for the specific comparisons using the Bonferoni t-test.

RESULTS AND DISCUSSION

Composition - Composition (Table 1.1) of various cuts of lamb carcasses was determined for the purpose of calculating the maximum amount of moisture picked up due to infusion. We were more concerned with the shoulder muscles because studies have shown that they usually have the highest moisture content compared to the leg and loin muscles (Paul et al., 1964). Because of variation in moisture content likely to be caused by difference in fat content (Spray et al., 1951), and the established inverse relationship between percent fat and moisture in the animal body (Batcher et al., 1962), moisture was compared on a MFF basis. The results (Table 1.1) showed that, in each case, the shoulder muscles retained more solution compared to the leg and loin muscles. The higher amount of moisture (MFF) in the shoulder (1.7% difference) and loin (1.1% difference) muscles of infused animals (average of NCa and WCa values) compared to control was significant ($P < .05$). The difference in MFF between leg muscles (.4%) of infused animals and that of control was not significant ($P > .05$). The difference in percent retained moisture in the different muscles of infused animals could result from the fact that the infusion site is closer to the shoulder area and farthest from the leg or because of the downward movement of

TABLE 1.1 EFFECT OF INFUSION ON THE COMPOSITION OF OVINE MUSCLES

Carcass Part	Infusion Treatment			SEM	Upper Limit 95% C.I.
	Ctr	NCa	WCa	±	
LEG					
Moisture	71.98[a]	72.50[a]	72.66[a]	.66	1.92
Fat	7.10[a]	7.02[a]	6.64[a]	.77	2.26
Protein	19.43[a]	19.14[a]	19.12[a]	.26	.77
MFF	77.47[a]	77.97[a]	77.82[a]	.17	.49
LOIN					
Moisture	70.53[a]	72.36[a]	71.61[a]	.70	2.05
Fat	7.54[a]	6.78[a]	7.07[a]	.74	2.16
Protein	20.24[a]	19.59[a]	19.84[a]	.27	.81
MFF	76.27[a]	77.61[b]	77.06[b]	.27	.80
SHOULDER					
Moisture	74.44[a]	76.62[b]	76.27[b]	.52	1.53
Fat	5.23[a]	4.59[a]	4.77[a]	.43	1.27
Protein	18.57[a]	17.07[b]	17.37[b]	.29	.85
MFF	78.54[a]	80.29[b]	80.09[b]	.28	.82

Values are means of 16 measurements (n = 8).
Ctr = Control, NCa = no $CaCl_2$, WCa = with $CaCl_2$, MFF = moisture fat free,
C.I. = confidence interval of difference between two means.

[a,b] Any two means in the same row with the same superscript are not significantly different ($P < .05$) by Dunnett's t-test.

solution by gravity during the 24 h holding period. Within a two-sided 95% upper limit of confidence interval, the greatest difference in MFF between infused and control carcasses is 2.5% ($P < .05$) in the shoulder (supraspinatus) muscle. This value was obtained by adding .82% to the difference between infused carcass MFF and that of control. If percent moisture instead of MFF was used, the difference would have been 3.5%. Therefore, the importance of using MFF to remove the influence of fat levels is underscored.

Percent fat did not differ ($P > .05$) between infused and control samples in any of the three cuts. Higher ($P < .05$) protein percentage was observed in the shoulder muscles of control animals compared to the infused ones. However, no difference ($P > .05$) in percent protein was found between the leg and loin muscles of infused and control animals. The difference in protein percentage between infused and control animals is likely due to dilution effect caused by the higher retained moisture in the shoulder muscles. To our knowledge no similar studies have compared the distribution of solution in infused carcasses.

Water-Holding Capacity (WHC) - Fresh lean meat at slaughter contains about 75% water. Eighty-five to 95% of this water is held within the muscle fiber in dynamic equilibrium with the remaining 5-15% plasma fluid outside the fiber walls. This amount of water is subsequently subjected to considerable variation resulting from the changes that

occur during processing through drip, cooking or evaporation. The retention of this water is of importance to the meat industry (Penny, 1975; Offer and Trinick, 1983; Paterson et al., 1988). The ability of meat to retain its moisture or added moisture is termed WHC, and is used to describe the phenomenon of drip, thaw drip and cooking losses (Hamm, 1986; Kauffman, 1986).

The data in Table 1.2 illustrate the effects of infusion on WHC as measured by drip, thaw drip and cooking losses in lamb leg steaks. In general, infused samples had higher losses compared to control samples, while frozen and thawed samples had higher losses compared to refrigerated samples. No difference ($P > .05$) between samples was observed under refrigerated conditions. However, in dealing with moisture losses, lack of significant statistical difference may not necessarily mean lack of significant dollar losses if the volume of animals processed in slaughter houses is considered. For instance, while the difference between WCa and Ctr (Table 1.2) for refrigerated steaks was not significant, WCa steaks had about .25% more drip than Ctr. The importance of accumulated drip in retail packages has been reported (Paul et al., 1964; Farouk et al., 1990).

The reason for the difference observed between treated and control samples in terms of all losses could be attributed to the increased moisture retained in infused samples which

TABLE 1.2. EFFECT OF INFUSION ON DRIP, THAW DRIP AND COOKING LOSSES OF OVINE MUSCLES

Measure	Treatment Means			SEM
	Ctr	NCa	WCa	±
Drip Loss (%)				
Refrigerated	1.25[a]	1.28[a]	1.53[a]	.16
Frozen	2.21[a]	3.90[b]	3.84[b]	.16
Cook Loss (%)				
Refrigerated	32.73[a]	32.76[a]	33.79[a]	.48
Frozen	36.09[a]	37.80[b]	37.86[b]	.48

Values are means of 16 measurements (n = 8).
Refrigerated (4°C), Frozen (-25°C).

[a,b] Two means in the same row bearing the same superscript are not significantly different at $P < .05$ for drip loss, and $P < .1$ for cook loss, by Bonferoni t-test.

may not have been fully incorporated within the muscle fibers. The pressure (3.2 kg/cm^2) employed might have ruptured the muscle microstructure contributing to the ease with which the muscle lost water. Some of the factors which affect WHC like freezing, length of frozen storage, thawing and packaging methods are well documented (Anon and Calvelo, 1980; Jeremiah et al., 1981a, b; Moore, 1990).

Another important reason for the higher moisture losses in infused carcasses may be the rapid rate with which pH was lowered in the infused carcasses compared to the controls (refer to figure 4a and 4b in chapter 3). The reason for the rapid drop in pH is discussed in chapter 3, however, the influence of the infusion process and the added ingredients could not be discounted (Pearson et al., 1973; Hamm, 1977). The lower pH at higher temperature may be detrimental to the muscle proteins which are responsible for the WHC of meats (Judge et al., 1989). Bhattacharga et al. (1988) reported increased drip loss with increased temperature in ground beef patties which they attributed to denaturation of proteins. The higher losses in WCa animals could be due to the calcium-induced shortening of muscles as explained in Chapter 4, or the destabilizing effects of Ca^{2+} on proteins or reduction of interfilament distance caused by the adsorption of Ca^{2+} in the muscles (Von Hippel and Schleich, 1969; Offer and Trinick, 1983) in addition to acceleration of glycolysis. Higher cooking losses in lamb and beef carcasses due to infusion of

.3M $CaCl_2$ has been reported by Koohmaraie et al. (1990).

Although some of the ingredients used in this study such as phosphate and glycerin were reported to increase WHC (Lacroix and Castaigne, 1985; Anjaneyulu, 1990; Bernthal et al., 1991), no such effect was observed in this study. This may result from the lower concentration used in this study or because any effect of such ingredients might have been masked by an opposing effect of other factors related to the infusion process.

Color - The Hunter L, a and b values for infused and control ovine carcasses are shown in Table 1.3. Hunter L, a and b are indications of lightness, redness and yellowness, respectively (Bhattacharya et al., 1988; Brewer and Harbers, 1991). Results of double split plot with repeated measure ANOVA indicated that for "L" values, there was no effect of treatment ($P > .05$). There were significant effects of storage ($P < .01$) and time ($P < .01$), but no interaction ($P > .05$) between treatment and time. Interactions between treatment and storage, and storage and time were significant ($P < .01$). For "a" values, treatment ($P < .1$), storage ($P < .01$) and time ($P < .01$) effects were significant. There were interactions between treatment and storage ($P < .2$), treatment and time ($P < .05$) and storage and time ($P < .01$). The effect of treatment ($P < .01$), storage ($P < .01$) and time ($P < .1$) were significant for "b" values with the only interaction

TABLE 1.3. RESULT OF HUNTER COLOR MEASUREMENTS OF LAMB LEG STEAKS FOR CONTROL AND INFUSED OVINE CARCASSES

Treatments	Storage Methods				SEM
	Refrigerated		Frozen		
	0 Day	7 Day	0 Day	7 Day	±
Ctr					
L	37.94	39.14	36.89	34.50	.32
a	10.71	8.74	11.19	11.71	.27
b	6.34	7.34	6.04	5.87	.20
NCa					
L	37.89	39.64	38.23	35.61	.32
a	11.25	8.49	11.43	11.09	.27
b	7.60	8.36	7.63	7.26	.20
WCa					
L	37.93	39.45	38.79	35.01	.32
a	10.64	7.54	10.15	10.09	.27
b	7.24	7.71	7.06	6.38	.20

Values are means of 48 measurements, n = 8.
Ctr = Control, NCa = no $CaCl_2$, WCa = with $CaCl_2$.
L = lightness, a = redness, b = yellowness.
Refrigerated (4°C), Frozen (-25°C).

(P< .01) existing between storage and time in this measure. Due to the various interactions shown in these data, results were analyzed in two parts: 1) influence of treatments on color (Table 1.4) and 2) the effect of storage and time on the color of ovine muscle (Table 1.5).

Comparing the effects of infusion on color in the various treatments (Table 1.4), no effect of treatment on "L" values of refrigerated steaks was evident, but in frozen steaks, "L" values were higher (P < .01) for treated samples. Treated samples were lighter (P < .05) than controls at d0, but at d7, only NCa was lighter than controls (P < .05). The "a" values were lower (P < .05) in WCa compared to NCa and Ctr for both refrigerated and frozen storage. At d0, NCa had higher (P <.01) red color readings compared to WCa, and at d7 both NCa and Ctr had higher (P < .01) "a" values than WCa. Infused samples were more yellow (P < .01) than control samples irrespective of method and time of storage. Among the infused samples, NCa had higher (P < .01) "b" values than WCa. Infusion with $CaCl_2$ was found to increase deterioration in the color of meat compared to the other treatments.

The lighter color of infused samples may result from an increase in light scattering or dilution of pigments in the muscles. The increase in scatter may also be the reason for the higher "b" values observed (Macdougall, 1982). The increase in yellowness may be due simply to increase in

TABLE 1.4. EFFECT OF INFUSION ON THE COLOR OF LAMB LEG STEAKS: RESULT OF t-STATISTICS

Storage Method and Time	Treatment Contrast		
	Ctr vs NCa	Ctr vs WCa	NCa vs WCa
L = lightness			
Refrigerated	.72	.47	.25
Frozen	3.84	3.84	0
0 d	4.25	2.96	1.67
7 d	2.55	1.67	1.43
a = redness			
Refrigerated	.51	2.37	2.89
Frozen	.71	4.93	4.22
0 d	1.45	2.04	3.48
7 d	1.63	5.22	3.59
b = yellowness	9.31	4.97	4.33

Critical values, Bonferoni t-statistics, .05 = 2.158, .025 = 2.435, .005 = 3.007, for comparison with table values.
Ctr = Control, NCa = no $CaCl_2$, WCa - with $CaCl_2$.
Refrigerated (4°C), Frozen (-25°C).

lightness as shown by the simple correlation analysis (Table 1.6). The lower red color observed in infused samples after storage could result from faster accumulation of metmyoglobin in these muscles resulting from the greater metmyoglobin reducing activity of the muscles earlier during the storage compared to non-infused muscles (Ledward, 1985). Differences in the rate of glycolytic changes as measured by pH and temperature decline (refer to chapter 3) might explain the faster rate of color deterioration in infused samples compared to controls. The rate of metmyoglobin accumulation was shown to be affected by time, temperature and pH history of a muscle (Hood, 1980; Ledward, 1985). According to Renerre and Labas (1987), the biochemical factors that influence the rate of discoloration of meat include: myoglobin autoxidation, enzymic ferrimyoglobin reduction and oxygen consumption rate. In samples infused with $CaCl_2$, "a" values may have been lowered due indirectly to the promotion of lipid oxidation (St. Angelo et al., 1991). Brewer and Harbers (1991) observed an increase in lipid oxidation accompanied by a decrease in red color ("a" values) in ground pork. Wu et al. (1991) reported lower Hunter color values in hot boned pork roast injected with solution containing glucose, NaCl, polyphosphates and KCl compared to controls. The reason for the higher red color values in freshly cut samples infused without $CaCl_2$ is not known. Table 1.5 shows the effect of

TABLE 1.5. EFFECT OF STORAGE AND TIME ON THE COLOR OF LAMB LEG STEAKS: RESULTS OF t-STATISTICS

Contrast	Hunter L, a, b Parameter		
	L	a	b
Ref vs Froz (0 d)	-	.27	.29
Ref vs Froz (7 d)	-	12.32	8.0
0 d vs 7 d (Ref)	5.75	11.86	4.6
0 d vs 7 d (Froz)	11.27	0.18	2.5
Ref vs Froz (Ctr)	8.94	6.37	-
Ref vs Froz (NCa)	5.83	5.14	-
Ref vs Froz (WCa)	5.57	3.82	-

Critical Values, Bonferoni t-Statistics, .05 = 2.158, .025 = 2.435, .005 = 3.007, for comparison with table values.

Ctr = Control, NCa = no $CaCl_2$, WCa = with $CaCl_2$.
L = lightness, a = redness, b = yellowness.
Ref = refrigerated (4°), Froz = frozen (-25°C).

storage temperature and time on Hunter L, a and b values. Results indicate that frozen samples had higher ($P < .01$) "a" values and lower ($P < .01$) "b" values compared to refrigerated samples. Holding samples in lighted refrigerated display for 1 wk lowered ($P < .01$) "a" values and increased ($P < .01$) "L" and "b" values. On the other hand, frozen storage in the dark did not affect ($P > .05$) "a" values, but lowered ($P < .01$) "L" and "b" values of the steaks. Frozen steaks had redder color ($P < .01$) and less yellowness ($P < .01$) for all treatments than those refrigerated. The effect of frozen storage on "L" and "a" values of steaks were in the order: Ctr> NCa > WCa.

The more desirable color (higher "a" values) observed in frozen steaks could result from reduced metmyoglobin accumulation caused by the activity of the metmyoglobin reducing enzymes at low temperature (Ledward, 1985). In addition to the higher temperature of storage, display lighting may have contributed to the faster deterioration of the red color in refrigerated meats (Kropf, 1980). The lower "L" values in frozen and thawed samples could not be explained. The "b" values were lower as a result of the positive correlation that exists between "L" and "b" values (Table 1.6). The lower "L" and "b" values of frozen steaks was in agreement with the findings of Bhattacharya et al. (1988) who reported that L, a and b values were lowered in frozen ground beef irrespective of temperature and time of

TABLE 1.6. SIMPLE CORRELATION COEFFICIENTS AMONG HUNTER L, a and b VALUES OF LAMB LEG STEAKS

Storage/Time	Treatment					
	Control			Infused		
	L & a	L & b	a & b	L & a	L & b	a & b
Fresh (0 d)	-.63	+.15	-.60	-.03	+.74	-.03
Ref (7 d)	-.76	+.68	-.63	-.08	+.58	-.18
Froz (7 d)	-.94	+.48	-.63	-.44	+.75	-.74

Ref = refrigerated, Froz = frozen.

L = lightness, a = redness, b = yellowness.

storage. Higher "L" and "b" values observed in refrigerated samples may have been caused by the decrease in "a" values. Brewer and Harbers (1991) reported a high negative correlation between "L" and "a" with a low correlation between "a" and "b" values in frozen ground pork. The results of our study (Table 1.6) supports this finding in part. The results show that the relationships between various color measurements were affected by treatments. High negative correlations exist between "L" vs "a" and "a" vs "b" values in control samples across storage, but in infused samples these relationships were observed only in frozen samples. High correlations exist between "L" and "b" in all treatments, storage and time, except in control samples at d0.

Conclusion - Carcasses could be infused with a volume equal to 10% of the animal live weight without a difference in MFF greater than 2.5% (control vs infused). Differences in the distribution of solution should be taken into consideration when different muscles are to be compared. Frozen storage of infused samples lowers their WHC, whereas lighted refrigerated storage leads to faster deterioration of muscle color. Infusion of the ingredients used in this study without $CaCl_2$ was beneficial to the color of freshly cut meat. Inclusion of calcium in the infusion medium is not recommended because of the effects on WHC and color. Further studies are needed to determine the effect of individual or combination of

ingredients on the color and WHC of meat with a view to exploiting the potential of the infusion process.

CHAPTER 2

THE EFFECT OF POSTEXSANGUINATION INFUSION OF BEEF ON COMPOSITION, TENDERNESS AND FUNCTIONAL PROPERTIES

ABSTRACT

Ninety culled dairy cows were used in this study and were paired by weight and conformation similarity. Forty-five cows were arterially infused immediately after bleeding with 10% volume by weight of a solution composed of dextrose (.23%), glycerin (.21%), a phosphate blend (.14%) and maltose (.1%). The remaining cows (45) served as control. In infused carcasses, quantity of solution was in the following order: supraspinatus, chuck (2.32%) > longissimus, loin (.32%) > semitendonosus, round (-.18%). Accordingly, percentage of protein, ether extractable fat and protein fat free (PFF) amounts were lowered ($P<.05$) and percentage of moisture, moisture protein ratio (M:P) were raised ($P<.05$) in the supraspinatus muscles. Tenderness (WBS, $P<.01$) and protein extractability (EP, $P<.15$) were improved. No difference was observed in water-holding capacity (WHC) between infused and control carcasses. Percentage of moisture fat free (MFF, r =

+.85) and protein fat free (PFF, r = -.97) were highly correlated to M:P. Moisture percentage of the fat free tissue (MFF) was shown to be a more consistent indicator of added moisture in infused whole carcasses compared to M:P and PFF. Very low correlations were observed between WBS, percent moisture, percent WHC and ether extractable fat. Within 99% confidence interval, the 3% ceiling for added moisture stipulated by the USDA was not exceeded. The economics of the infusion process to the beef industry is discussed.

INTRODUCTION

Tenderness is one of the important palatability characteristics of meat (Calkins and Seideman, 1988; Cross,1987). Considerable amount of time and resources have been expended in an attempt to improve tenderness with varying degrees of success. Tenderness is particularly important in beef compared to pork and lamb (McGill, 1981). However, increased nutritional concern by the consumer is one factor causing a decline in beef sales volume. Additionally, the continued practice of aging carcasses for 8 to 14 d to achieve tenderness (Koohmaraie et al., 1986) has made the industry the most energy-intensive of all food industries (Henrickson, 1981). It is, therefore, necessary for the industry to devise ways of improving tenderness in a shorter period of time so as to reduce energy and labor costs. This much needed savings can be used to make the industry more competitive.

Recent reports indicate some progress is being made in regard to accelerating the process of aging. Koohmaraie et al (1988b, 1990) demonstrated that, in ovine carcasses, postmortem storage beyond 24 h for the purpose of achieving tenderness can be eliminated by the infusion of a calcium chloride solution. Ingredients such as maltose, dextrose, glycerine, polyphosphates and water have earlier been used in various combinations as tenderizing blends (Karmas, 1970). The

role of these ingredients in the improvement of tenderness, water-holding capacity and color and in increasing the rate of glycolysis have been reported (Whiting, 1987; Pearson and Tauber, 1984; Trout and Schmidt, 1983; Hamm, 1977; Pendleton, 1970; Anon, 1968). In a separate study already discussed in chapter 1, infusion of dextrose, maltose, glycerin and polyphosphates significantly increased the rate of glycolysis in treated lambs compared to controls. There is a need to know whether this process meets the USDA regulation for added moisture in beef and what effect it has on certain functional properties which are likely to affect the quality of meat for processing. Such properties as water-holding capacity (WHC) and protein extractability (EP) were reported to be very important (Smith, 1988, 1991; Wismer-Pedersen, 1987).

This study was designed to investigate the effect of infusing maltose, dextrose, polyphosphate and glycerin on composition, tenderness and functional properties of beef.

MATERIALS AND METHODS

Animals

For this experiment, a total of 90 utility holstein cows (45 pairs, 380 to 750 kg live weight) were divided into two groups: 1) control (cows slaughtered and dressed according to normal procedures in Abbyland Meats slaughter plant, Abbyland Processing, Inc., Abbotsford, WI.) and 2) infused with 10% live wt of solution containing .1% maltose, .21% glycerin, .23% dextrose, .14% blend of sodium and potassium tripolyphosphates. The composition of the infusion solution was determined through preliminary trials conducted. Cows were sorted and paired by similarity in weight and conformation before slaughter.

Infusion and Tissue Sampling

After the cows were stunned, they were hoisted and then bled via the right and left jugular vein. One branch of carotid artery was exposed and a sanitized nozzle of an infusion pump (MPSC, Inc., Eden Prairie, MN.) was inserted and secured. Solution was pumped (3.2 kg/cm^2) into the artery until 10% by weight of the animal was dispensed. After infusion was completed, carcasses were dressed, halved, inspected and transferred to a holding cooler (2 to 10°C). Twenty four hours after slaughter, representative samples of the chuck (m. supraspinatus), loin (m. longissimus) and round

(m. semitendonosus) were taken for proximate analysis. Part of the longissimus was also taken for tenderness, water-holding capacity (WHC) and protein extractability (EP) measurements.

Proximate Analysis

Percentage of moisture, fat and protein were measured as described in the AOAC (1990) methods to determine the distribution of solution in the carcasses. The result obtained was used to calculate percent moisture fat free, MFF ([moisture %/(100 - fat %)] x 100) to adjust for variation in moisture caused by difference in fat content.

Water-Holding Capacity (WHC)

Percentage of cooking yield (weight after cooking/raw meat weight x 100) was used to measure WHC (Bernthal et al., 1989). Duplicate 5 g samples of ground muscle were weighed in Corex centrifuge tubes covered with glass marble and then placed in boiling water bath for about 20 min. After 15 min of cooking, the released juice was drained off and the meat blotted with a filter paper to determine the cook yield. Samples from 30 cows (15 pairs) were used for this measurement.

Protein Extractability (EP)

EP was measured using procedures of Saffle and Galbreath (1964) as modified by Bernthal et al. (1989) for extractable

protein. AOAC (1990) procedure was followed in determining total protein content of the meat. Duplicate 5 g samples were diluted with 20 ml of 1N NaCl solution. Samples were then homogenized for 6 sec (2 bursts, 3 s each), then centrifuged at 16,000 x g for 12 min. The supernatant was further centrifuged at the same speed for 12 min. The second supernatant was analyzed for protein concentration (AOAC, 1990). EP was the amount of protein in the supernatant expressed as percent of total protein. Samples from 30 cows were used in this measurement.

Shear Force Determination

Ninety steaks, 3.7 cm thick, were vacuumed packed and cooked in a smokehouse oven (Drying System, Inc., Chicago, IL.) to an internal temperature of 70^{0}C. Steaks were chilled overnight at 2 to 4°C before they were cored. Five 1.27 cm cores were cut such that the cores were parallel to the fiber direction of the muscle for Warner-Bratzler shear (WBS) force determinations. A total of 10 measurements were taken from each steak.

Statistical Analysis

Cows were blocked on the basis of all controllable factors such as live weight, body conformation, breed, and age. Within a block (pair) cows were randomly assigned to the

two treatments (Gill, 1978a). Hypothesis was tested using paired t-test (Ecosoft, 1984). One-sided upper limit of confidence interval (at 99% level) was used to determine the maximum moisture pickup possible in the infused samples compared to controls.

RESULTS AND DISCUSSION

Composition

The changes in composition in the three locations in the carcass are shown in Table 2.1. Result indicates that, overall, the supraspinatus (chuck) muscle retained more solution compared to the longissimus (loin) and the semitendonosus (round) muscles in infused carcasses. The amount of moisture (MFF) in the supraspinatus (SS) muscle of infused carcasses was 2.22% higher than in control. No difference ($P>.05$) was found between the longissimus (L) (.34% difference in MFF) and the semitendonosus (ST) (-.18% difference in MFF) muscles of infused and control carcasses. The higher retained moisture in the SS may be due to the proximity of the muscle to the infusion site or because of the downward movement of solution by gravity during the chilling period (Chapter 1). In contrast to the lamb data reported in chapter 1, the result of this study did not show any significant difference in MFF in the L and none in the ST muscles. The position of carcasses during the infusion process could explain the difference between the two studies. Whereas lambs were infused in a cradle, the beef carcasses were infused hanging on the rail. For this reason, it was probably difficult for the solution after reaching the beef round area to remain there for long. Within a 99% confidence

TABLE 2.1. EFFECT OF INFUSION ON COMPOSITION OF DIFFERENT CUTS OF BEEF

Prime Cut	Measure[a]	Treatment Mean[b]				P-value
		Ctr	SD	Inf	SD	
Chuck	Moisture	73.22	2.7	76.51	2.6	*
	Fat	4.98	3.3	3.49	2.5	*
	Protein	20.43	.9	18.35	1.2	*
	MFF	77.05	.9	79.27	1.3	*
	PFF	21.51	1	19.02	1.2	*
	M:P	3.59	.2	4.17	.31	**
Loin	Moisture	71.98	4	72.86	.81	
	Fat	5.73	4.8	5	3	
	Protein	21.63	1.3	21.4	1	
	MFF	76.35	1.1	76.7	1	
	PFF	22.97	1.3	22.54	1.1	
	M:P	3.34	.23	3.41	.2	
Round	Moisture	73.45	2.2	73.33	1.9	
	Fat	4	2.7	3.88	1.9	
	Protein	21.65	1	21.91	.8	
	MFF	76.52	1	76.28	.8	
	PFF	22.55	1.1	22.8	.9	
	M:P	3.4	.2	3.35	.2	

[a] MFF = Moisture Fat Free, PFF = Protein Fat Free, M:P = Moisture Protein Ratio, Ctr = Control, Inf = infused

[b] Values are means of 90 (n = 45) measurements.the muscles in

* $P < .05$.

** $P < .01$.

limit (Table 2.2), MFF of the infused carcasses did not exceed a difference of + 2.8% compared to controls. Based on the data obtained (Table 2.2), the greatest difference in MFF likely is 2.8%, .96% and .28% in the SS, L and ST, respectively. In

TABLE 2.2. MAXIMUM DIFFERENCE IN MFF IN THREE MUSCLES BETWEEN INFUSED AND CONTROL BOVINE CARCASSES

Muscle	MFF difference (Inf - Ctr)	99% Upper limit of confidence interval
Supraspinatus	2.32	2.8
Longissimus	.34	.96
Semitendonosus	-.18	.28

terms of potential weight gain by individual muscles, these differences in MFF translate to between 6 and 11% for SS, 1 to 2% for the L and less than 0 and 0% for the ST, depending upon method of computation. Results (Table 2.1) showed that percent fat and protein were lower ($P<.05$) in the chuck muscle of infused carcasses compared to the controls. However, percent protein and fat did not differ ($P>.05$) with control in the loin and the round of infused carcasses. The reason for the observed difference in the chuck muscle could be because of dilution effects caused by higher moisture in the chuck muscle. Similar observations were made in infused lamb

carcasses as was discussed in chapter 1.

The MFF values were used to indicate the relative added moisture (Table 2.1) in order to remove variation in percent moisture which might be caused by the variations in percent ether extractable fat. In addition when MFF was compared to M:P as a measure of added moisture, the added moisture calculated according to the formula reported by USDA (1986) gave some negative results. For instance, when a protein multiplier of 3.8 was used, the amounts of added moisture based on that M:P were 6.78%, -8.46% and -9.92% in the chuck, loin and the round of infused carcasses, respectively. There was a wide range of M:P ratios observed in this group of samples, possibly due to the variation in physiological condition of these carcasses attributable to handling and trucking. Another formula used was the measure of increased hot carcass weights in infused carcass due to treatment. According to the regulation, when beef cuts were infused for purpose of tenderization, the treated samples must not exceed the untreated ones by more than 3% measured by weight difference (USDA, 1986). Since our data showed that distribution of moisture in whole carcass infusion was not uniform, we felt the use of direct weight difference was not appropriate. For this reason, various cuts were compared separately and we found that the best way to eliminate the

variations likely to bias our calculations was to use MFF data in closely paired cows. Use of the mean PFF for controls to estimate added water in the tissues gave a very wide range of apparent water pickup (estimated weight gain) for individual samples (e.g., -10% to +9% for control SS and -1 to +21% for infused SS) and was judged not as suitable as MFF for that purpose. The use of PFF in control of added substance in cured pork products was discussed by Price and Stachiw (1985).

Water-holding capacity and Protein extractability

Water-holding capacity and EP values are shown in Table 2.3. The results indicate no difference ($P>.05$) between infused and control carcasses in WHC. However, the infused samples had higher amount of EP ($P<.15$). In the lamb study discussed in chapter 1, no difference was found in WHC between infused and control lamb carcasses refrigerated 1 wk. Protein extractability might have been increased by the added ingredients in the infusion solution. Bernthal et al. (1991) indicated that addition of tetrasodium pyro-phosphate (TSPP) to pre-rigor beef muscle homogenates increased protein extractability.

Tenderness

The effect of infusion on tenderness as measured by Warner-Bratzler shear force (WBS) is shown in Table 2.3. The

result indicates that WBS was improved by 13% ($P<.01$) as a result of the infusion process. The improved tenderness of infused samples is corroborated by the higher protein extractability (EP) observed in the same samples (Table 2.3). The increase in EP of tender beef was attributed to the weakening of fibrous protein linkages (Davey and Gilbert, 1968). Increase in EP with tenderness was also observed in turkey breast muscle (Landes et al., 1975). The lower WBS of infused samples could be as a result of the enhancement of the normal proteolytic processes attributale to the added ingredients in the infusion solution or possibly due to the disruption of the muscle structure caused by the shear volume of and pressure (3.2 kg/cm^2) with which the solution was introduced into the carcasses. The added phosphate among the ingredients may contribute to the solubilization of actomyosin (Lewis et al., 1986; Pearson and Tauber, 1984). Phosphates could also chelate divalent cations thereby reducing the interfibrillar association, consequently reducing toughness (Trout and Schmidt, 1983). The role of phosphates in accelerating glycolytic changes was reported by Hamm, 1977. If indeed glycolytic changes were accelerated in the beef carcasses as was shown in infused lamb carcasses (Chapter 3), then the early completion of rigor in the infused samples could have encouraged the earlier commencement of aging

TABLE 2.3. EFFECT OF INFUSION ON TENDERNESS, WATER-HOLDING CAPACITY (WHC) AND PROTEIN EXTRACTABILITY (EP) OF BEEF[a]

Measure	Mean Values		P-value
	Control	Infused	
Shear force, kg	8.71 ± 2.1	7.64 ± 1.4	.006
WHC, %	78.90 ± 2.9	78.29 ± 3.1	.342
EP, %	41.96 ± 3.6	43.48 ± 3.4	.152

[a] Values are means of: 450 (n = 45) measurements for shear force
30 (n = 15) measurements for WHC
30 (n = 15) measurements for EP

process in these samples compared to the controls. The infusion process itself could have stimulated some proteolytic enzymes leading to the greater tenderness observed in this study. Koohmaraie et al. (1988b, 1990) demonstrated that infusion of 0.3M $CaCl_2$ significantly increased tenderness in lambs. They (Koohmaraie et al., 1988; 1990) attributed the improved tenderness to the activation of calcium dependent proteases (CDP's). Nevertheless, further studies are needed to ascertain the role of these ingredients individually and in combination on the proteolytic enzyme activities, particularly CDP's and cathepsins. There is also the need to look at how the process affects the microstructure of muscles from infused carcasses.

Correlations

Results of correlation coefficients (Table 2.4) showed a high inverse relation between moisture and fat measured in the chuck of both infused and control animals. The same observation was made by several other authors (Ramsey et al., 1990; Carpenter and King, 1965; Batcher et al., 1962). Significant correlations were also shown between MFF and PFF (-.4), MFF and M:P (+.85), and PFF and M:P (-.97). The implication of this result is that MFF can be used in place of PFF and M:P in determining the amount of added moisture as

TABLE 2.4. SIMPLE CORRELATION COEFFIENTS BETWEEN VARIOUS MEASUREMENTS OF BEEF CHUCK AND LOIN MUSCLES

Muscle Cut	Trait[a,b]	r
Chuck	% Moisture vs % Fat	-.88
	MFF vs PFF	-.40
	MFF vs M:P	+.85
	PFF vs M:P	-.97
Loin	% Moisture vs Shear Force	+.21
	% Fat vs Shear Force	-.24
	WHC vs Shear Force	-.04
	EP vs Shear Force	+.06
	WHC vs EP	-.50
	% Protein vs EP	-.11

[a] n = 90 for all traits, except WHC and PE (n = 30).
[b] from table 1.

previously discussed. A USDA (1984) study indicated a close relationship between M:P and PFF. Our data showed very low correlations between % moisture and shear force (+.21), % fat and shear force (-.24), % protein versus EP (-.11), WHC and shear force (-.04) and EP versus shear force (-.06). The relationship between moisture and fat content to WBS reported in this study were consistent with what was reported by Ramsey et al. (1990). Their result showed that percent moisture and ether extractable fat were not closely associated to tenderness. However, our data did not agree with that of Hegarty et al. (1963a), that fibrillar protein solubility and

WHC were highly related to tenderness. The difference in our results may be attributed to the difference in the way the steaks used in the two studies were prepared and the length of time the steaks were aged before WBS was measured. Other studies supported our findings that WHC and protein solubility were not related to tenderness in bovine muscles (McClain and Mullins, 1969; Goll et al., 1964). The correlation (-.5) observed between WHC and EP in our study is in agreement with the value (-.62) reported by Sayre and Briskey (1963).

Economics of the Infusion Process

A lower cost of production in the beef industry may be a considerable factor in the competition of beef with chicken, pork and fish (Pearson, 1966). Considering the image of beef as "unhealthy", "fat", "heavy" and "calorie laden" (Allen, 1985), the proposition that the beef industry is one of the most energy intensive of all food industries (Henrickson and Asghar, 1985; Henrickson, 1981) and the relative high cost of beef at retail, processing steps taken to offset these negative factors may assist in making beef more competitive.

If the carcass infusion process were to be adopted in the beef industry, there would be opportunity to reduce cost factors or increase yield (Table 2.5.). If all or part of the yield increase could be translated into profit, increased revenue could offset processing cost disadvantages of the

industry. If not, consumers could benefit from decreased unit costs in the marketplace. There is potential for offsetting costs of energy input for chilling and possibly decreased cooler space in addition to tenderness improvement.

TABLE 2.5. YIELD OF CONTROL AND TREATED BEEF CARCASSES[a]

Measure	Mean Value	
	Control	Infused
Live wt, kg	536.1	534.8
Dressed wt, kg	253.6	265.3
Yield, %	47.3	49.6

[a] Values are means of 45 measurements (n = 45).

However, before the process is adopted across the industry, there is need to optimize the ingredients in the solution and to minimize color problems (as evidenced in chapter 1) and microbiological hazards.

Implications

Beef carcasses could be infused with a volume equal to 10% of their live weight with unequal pickup of added moisture

in various carcass parts and with no deleterious effect to the functional properties (WHC and EP) of the meat. In addition, tenderness and dressed carcass yield are improved by the process. More research is needed though, before the beef industry could realize the economic potential of the whole carcass infusion process.

CHAPTER 3

POSTMORTEM GLYCOLYSIS IN OVINE CARCASSES INFUSED POSTEXSANGUINATION

ABSTRACT

Twenty-four lambs were assigned to three treatment groups: 1) Control (Ctr), 2) Infused with 10% volume by weight of a tenderizing blend (NCa) and 3) NCa plus 0.015M $CaCl_2$ (WCa). Infusion lowered ($P<.05$) the temperature of carcasses over the first 3h postmortem (PM) compared to control carcasses. The rate of glycolysis was higher in infraspinatus muscle (IS) compared to longissimus muscle (LD). NCa-treated samples had significantly ($P<.05$) lower pH values over the first 6h PM in IS and over the first 12h PM in LD compared to Ctr and WCa. WCa had lower pH ($P<.05$) compared to Ctr at 3 to 12h PM. Concentration of lactic acid (LAC) in LD was higher ($P<.05$) in NCa compared to Ctr and WCa over the first 12h PM. In the same muscle, WCa had higher ($P<.05$) LAC compared to Ctr at 3h, 6h and 12 hr PM. In IS, NCa had higher ($P<.05$) LAC compared to Ctr and WCa over the first 6h and 12h PM. No differences ($P>.05$) in LAC existed between Ctr and WCa in IS. In both muscles, ATP levels were lower ($P<.05$) in NCa over the first 3 to 6h PM. WCa had lower ($P<.05$) ATP levels compared to Ctr at 3h PM in LD. In both muscles, glycolysis was completed within the first 6h PM in NCa; whereas in Ctr and WCa it took 12 to 24h for glycolysis to be completed. The rate of glycolysis was in the order: NCa>WCa>Ctr.

INTRODUCTION

Many quality factors of meat that are essential from the consumer point of view depends on the processes of postmortem glycolysis which is involved in the conversion of ATP replete muscle to ATP free meat (Jeacocke, 1984; Lee et al., 1978). The quality factors affected by glycolysis include: water-holding capacity, tenderness, color, flavor, juiciness, bacterial control, fermentation, aging, binding properties of comminuted and restructured meats and grading characteristics (Honikel and Potthast, 1991; Solomon, 1987; Dutson, 1983b; Marsh, 1981). The rate and extent of glycolysis is affected by: species difference (Lundberg et al., 1987), type of muscles (Honikel and Potthast, 1991; Bendall, 1978; Martin and Fredeen, 1974), location of muscle (Tarrant and Mothersill, 1977), electrical stimulation (Kondos and Taylor, 1987), comminution (Hamm, 1977), injection of $CaCl_2$ (Pearson et al., 1973), muscle mechanical damage due to injection process (Stanton and Light, 1990b), addition of sugars and phosphates (Bernthal et al., 1991; Young et al., 1988) and temperature (Jolley et al., 1981). Glycolysis is monitored by determining the rate of pH fall and/or the disappearance of ATP (Newbold and Small, 1985; Bendall, 1973; Bodwell et al., 1965).

The present study was undertaken to determine the influence of infusing a tenderizing blend on the postmortem glycolysis in ovine carcasses.

MATERIALS AND METHODS

Animals - The same animals used for the study described in chapter 1 were used for this study.

Infusion and Tissue Sampling - Lambs were infused as described in chapter 1. At the end of the infusion process, samples were taken at 0h (30 to 40 min) after the completion of infusion and at 1, 3, 6, 12 and 24h thereafter for measurement of ATP and lactic acid. The samples were frozen immediately in liquid nitrogen and stored at -75°C until analyzed.

pH and Temperature Determinations - Temperature and pH were measured (Solomat Modumeter® - Solomat Corp., 652 Glenbrook Road, Stamford, CT.) at 0h (30 to 40 min) after the completion of infusion process and at 1, 3, 6, 12 and 24h postmortem. The pH was measured in the longissimus dorsi and infraspinatus muscles, and the temperature was measured only in the longissimus muscle area.

ATP Measurement - ATP was measured as described by Strehler (1974) with some modification. One-half to 1g powdered sample was extracted for 10 min in 10ml boiling distilled water, instead of perchloric acid. The extract was filtered with Whatman No. 1 filter paper and .4ml of the extract or standard ATP solution was quickly mixed with .4ml reconstituted firefly latern extract (Sigma® Chemical Co., St. Louis, MO.) containing .05 potassium arsenate and .02M $MgSO_4$

at pH 7.4. The light intensity was measured after 5 s of mixing with an Aminco Photometer (American Instrument Co., Silver Spring, MD.) at wavelength of 562nm and 5x amplification. ATP concentration (μmol/g muscle sample) was calculated from a standard curve.

Lactic Acid Measurement - Lactic acid was measured as described by Noll (1984), except 4g powdered frozen samples were homogenized with 40ml cold 9% perchloric acid solution in a Waring blendor. 10N KOH was used to neutralize the extract and a Response® UV-VIS Spectrophotometer (Ciba Corning Diagnostics Co., Oberlin, OH.) was used to read the absorbance at 340nm.

Statistical Analysis - This experiment was designed as a split plot with repeated measure (time as a repeat measure) design (Gill, 1978b). The ANOVA table and the least significant difference (LSD) needed for comparisons were determined by SAS (1987). Due to significant ($P<.01$) interaction between time and treatment, contrast (non-orthogonal) was designed between treatments within each sampling time and for each sampling time within treatments separately for all parameters measured. The significance of difference was determined by Bonferoni t-test.

RESULTS AND DISCUSSION

Postmortem pH and Temperature Fall

Fig. 3 shows the chilling rate of carcasses for the different treatments employed in this study. Figs. 4a and 4b shows the fall in pH in the m. longissimus (LD) and m. infraspinatus (IS) muscles respectively. Muscle pH and temperature decreased continuously over the period of 24h postmortem (PM) as previously noted (Solomon, 1987; Bendall, 1978; Martin and Fredeen, 1974; Bodwell et al., 1965). The average rate of pH fall over the first 3h pm for all the treatments was .13 and .16 units/h in LD and IS, respectively. The rate of pH fall over the first 3h was higher in infused carcasses (NCa and WCa) compared to controls (Ctr), irrespective of muscle type. The result of t-statistics (Table 3.1) indicated that NCa had lower ($P<.05$) pH values compared to Ctr and WCa over the first 0 to 6h and 0 to 12h in IS and LD, respectively. WCa had lower ($P<.05$) pH values compared to Ctr over the first 3 to 6h and 6h in LD and IS, respectively. The difference in the rate of pH fall between LD and IS is in agreement with the earlier reports that glycolytic changes varied between different muscles on the same carcass (Bendall, 1978; Tarrant and Mothersill, 1977; Martin and Fredeen, 1974). The difference in the rate of pH fall beween infused and control carcasses could be attributed to the ingredients contained in the infusion solution and

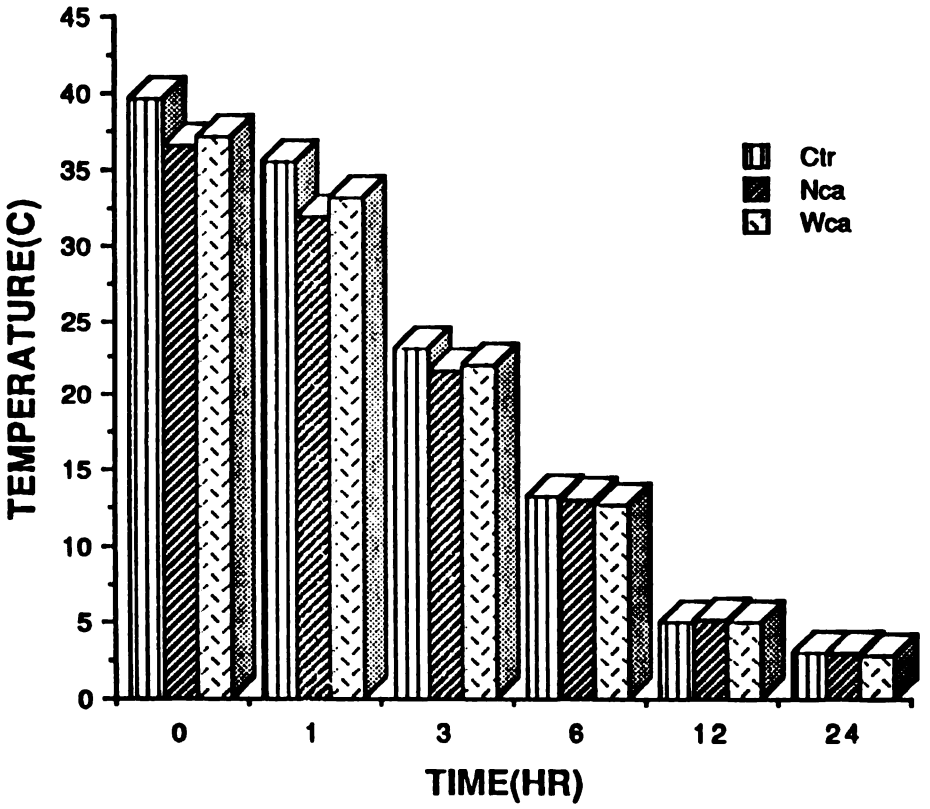


Fig 3. Chilling rate of control and infused ovine carcasses

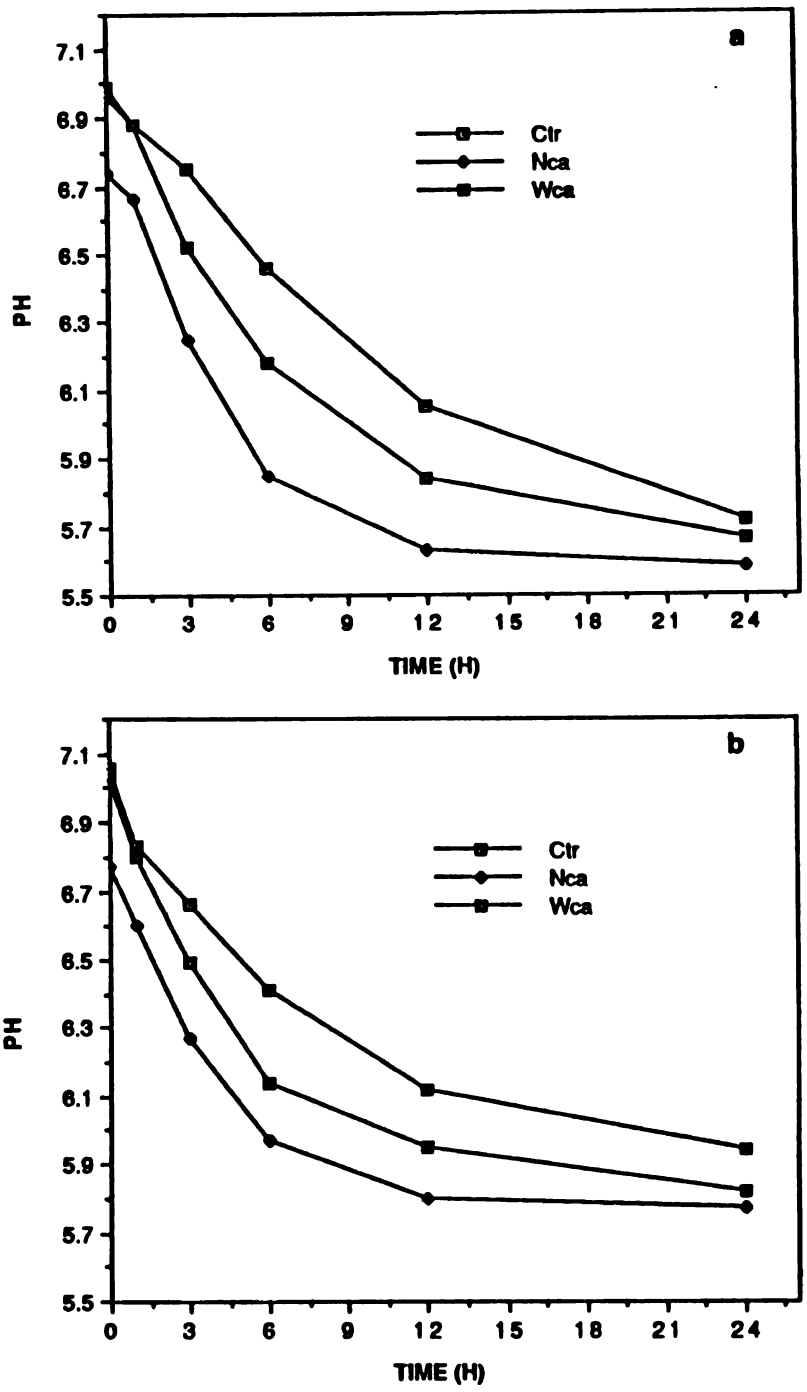


Fig 4a & b. Rate of fall of pH in ovine longissimus (a) and infraspinatus (b) muscles

TABLE 3.1. TREATMENT EFFECT ON pH VALUES WITHIN TIME (RESULT OF T-STATISTICS)*

Muscle	Time (h)	Contrast (Non-orthogonal)♦		
		Ctr vs NCa	Ctr vs WCa	NCa vs WCa
Longissimus	0	3.38	.46	3.85
	1	3.38	0	3.88
	3	7.69	3.54	4.15
	6	9.38	4.31	5.08
	12	6.46	3.23	3.23
	24	2.15	.92	1.23
Infraspinatus	0	4.04	.56	3.49
	1	3.21	.41	2.78
	3	5.44	2.37	3.07
	6	6.14	3.76	2.37
	12	4.46	2.09	2.09
	24	2.37	1.67	.70

* Calculated T values of the absolute difference between two treatments within time.
♦ Critical values, Bonferroni t-test, .05 =2.158, .025 = 2.435 for comparisons with table values.

Ctr = control, NCa = infused without $CaCl_2$, WCa = infused with $CaCl_2$.

possibly the stimulation of glycolytic enzymes as a result of the mechanical disruption of the musculature caused by the infusion process (Stanton and Light, 1990b; Trout and Schmidt, 1983; Hamm, 1977; Pearson et al., 1973). The probable cause of the retardation in the rate of pH fall in WCa compared to NCa treatments could be as a result of the effect of the $CaCl_2$ at the concentration added or the reaction products between $CaCl_2$ and the other infusion ingredients which might influence the glycolytic enzymes. Honikel and Hamm (1974) as cited by Hamm (1977) demonstrated that $CaCl_2$ at different concentrations may increase or inhibit the rate of glycolysis. Addition of .1M $CaCl_2$ either by injection or mixing in ground sheep muscle gave lower (but non significant) pH values compared to controls (Pearson et al., 1973). Other studies (Lundberg et al., 1987; Newbold and Small, 1985) demonstrated that postmortem pH values are good indicators of the rate of muscle glycolysis; therefore, based on the result of pH measurements obtained in this study, the rate of glycolysis in the three treatments is in the order: NCa>WCa>Ctr.

As shown in Table 3.2, the infusion process significantly ($P<.05$) lowered the carcass temperatures over the first 2 to 3h after the completion of the process as compared to the control carcasses. The lower temperature of the tap water used in the infusion in relation to the carcass temperature immediately after slaughter could be the reason for the lowering of temperature in the infused carcasses. It is

TABLE 3.2. TREATMENT EFFECT ON THE CARCASS TEMPERATURE WITHIN TIME (RESULT OF T-STATISTICS)*

Contrast (non-orthogonal)+	Time (h)		
	0	1	3
Ctr vs NCa	4.77	5.53	2.26
Ctr vs WCa	3.89	3.54	1.60
NCa vs WCa	.87	1.99	.67

* Calculated T values for the absolute difference in temperature of two treatments within time.

+ Critical Values, Bonferoni t-test, .05 = 2.158, 0.025 = 2.435, .005 = 3.0116, for comparison with table values.

Ctr = Control, NCa = infused without $CaCl_2$, WCa = infused with $CaCl_2$.

possible that the initial lowering of the carcass temperature might have retarded the rate of glycolysis in the infused carcasses. Higher temperatures have been associated with higher rate of glycolytic changes postmortem (Kondos and Taylor, 1987; Jolley et al., 1981).

Postmortem Changes in ATP and Lactic Acid

The mean ATP and lactic acid values are shown in figure 5a and 5b. The ATP values are in agreement with some values previously reported (Honikel and Potthast, 1991; Stanton and Light, 1990; Jolley et al., 1981) but were lower than other published values (Bendall, 1979; Bodwell et al., 1965). The fact that all comparisons were made with controls rendered the actual values less important. Initial ATP concentration is lower in IS compared to LD. The results in Table 3.3 indicated that in LD the ATP concentration were lower ($P<.05$) in NCa over the first 0 to 6h compared to WCa and at 0, 3 and 6h compared to Ctr. In the same muscle, WCa had lower ATP concentration compared to Ctr at 6h PM. In IS, the ATP concentration were lower ($P<.05$) in NCa compared to Ctr and WCa between 3to 6h and at 6h, respectively. The change in ATP concentration (Table 3.4) remained constant over the first 0 to 3h PM in Ctr and WCa in LD, but the change was constant only over the first 0 to 1 h in NCa in both LD and IS muscles. Significant changes ($P<.05$) in ATP concentration were observed in both muscles over the period between 3 to 12h PM in Ctr and

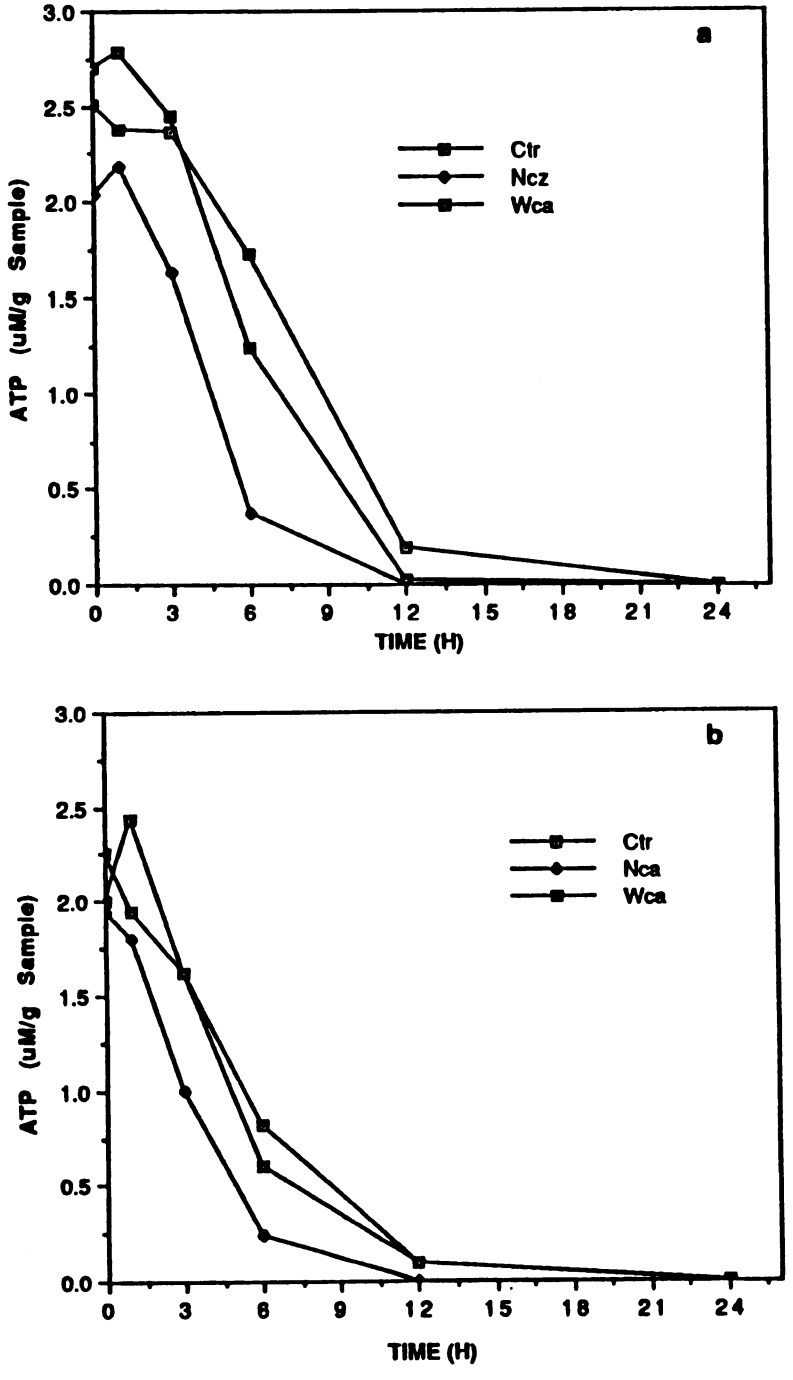


Fig 5a & b. Change in ATP concentration postmortem in ovine longissimus (a) and infraspinatus (b) muscles

TABLE 3.3. TREATMENT EFFECT ON THE DIFFERENCE IN ATP VALUES WITHIN TIME*

Muscle	Time (h)	Contrast (non-orthogonal)*		
		Ctr vs NCa	Ctr vs WCa	NCa vs WCa
Longissimus	0	.47	.19	.66
	1	.20	.39	.59
	3	.74	.08	.82
	6	1.36	.50	.86
	12	.19	.16	.03
	24	0	0	0
Infraspinatus	0	.06	.26	.32
	1	.64	.50	.14
	3	.62	0	.62
	6	.57	.21	.36
	12	.10	0	.10
	24	.01	.01	0

* Absolute differences between the means of two treatments obtained by calculating difference between two values found in Tables 3.1 and 3.2.

* Bonferoni t-tests at 5% level of significance. Least significant difference (LSD) for longissimus = .42 and for infraspinatus = .39 for comparison with table values.

Ctr = control, NCa = infused without $CaCl_2$, WCa = infused with $CaCl_2$.

TABLE 3.4. CHANGES IN ATP VALUES WITH TIME WITHIN TREATMENTS*

Muscle	Contrast (non-orthogonal)+	Treatments		
		Ctr	NCa	WCa
Longissimus	0 h vs 1 h	.13	.14	.07
	1 h vs 3 h	.01	.55	.32
	3 h vs 6 h	.64	1.26	1.22
	6 h vs 12 h	1.54	.37	1.20
	12 h vs 24 h	.19	0	.03
Infraspinatus	0 h vs 1 h	.44	.14	.32
	1 h vs 3 h	.82	.80	.32
	3 h vs 6 h	.81	.76	1.02
	6 h vs 12 h	.71	.24	.50
	12 h vs 24 h	.09	0	.10

* Absolute difference between the mean values of two times within a treatment obtained by calculating differences between the values in Tables 3.1 and 3.2.

+ Bonferoni t-test at 5% level of significance. The LSD for longissimus and infraspinatus for comparison with table values are 0.42 and 0.39, respectively.

Ctr = control, NCa = infused without $CaCl_2$, WCa = infused with $CaCl_2$.

WCa, but no change (P>.05) in ATP concentration were observedin NCa after 6h PM. The reasons for the lower ATP concentration observed in NCa compared to the other treatments could be because of the faster rate of glycolysis in NCa as noted previously. The rate of hydrolysis of ATP was reported to be the driving force and the determinant of the rate of glycolysis (Bendall, 1973). The two phases of ATP fall (constant concentration over the first 0 to 3h and the falling phase over the remaining periods) observed in this study agrees with what was previously reported (Jolley et al., 1981; Bendall, 1973).

The results of lactic acid measurement (figure 6a and 6b) indicate that the concentration of lactic acid (LAC) increased with time irrespective of muscle or treatment. The increase in LAC with time was also observed by Bendall (1973) and Bodwell et al. (1965). LAC in the LD (Table 3.5) was higher (P<.05) in NCa compared to Ctr and WCa over the first 12h PM. In the same muscle, WCa had higher (P<.05) LAC at 3, 6 and 24h PM compared to Ctr. In IS muscle, NCa had higher LAC over the period between 0 to 6h and 0 to 12h compared to Ctr and WCa, respectively. WCa differed with Ctr only at 0h. In LD, LAC continues to change (Table 3.6) in Ctr and WCa over the 24h period PM, whereas no significant (P>.05) change in LAC took place in NCa after 6h PM. LAC did not change (P>.05) in NCa samples in IS after 6h as was observed in LD; however, the change in LAC continued in Ctr and WCa beyond 6h PM in this

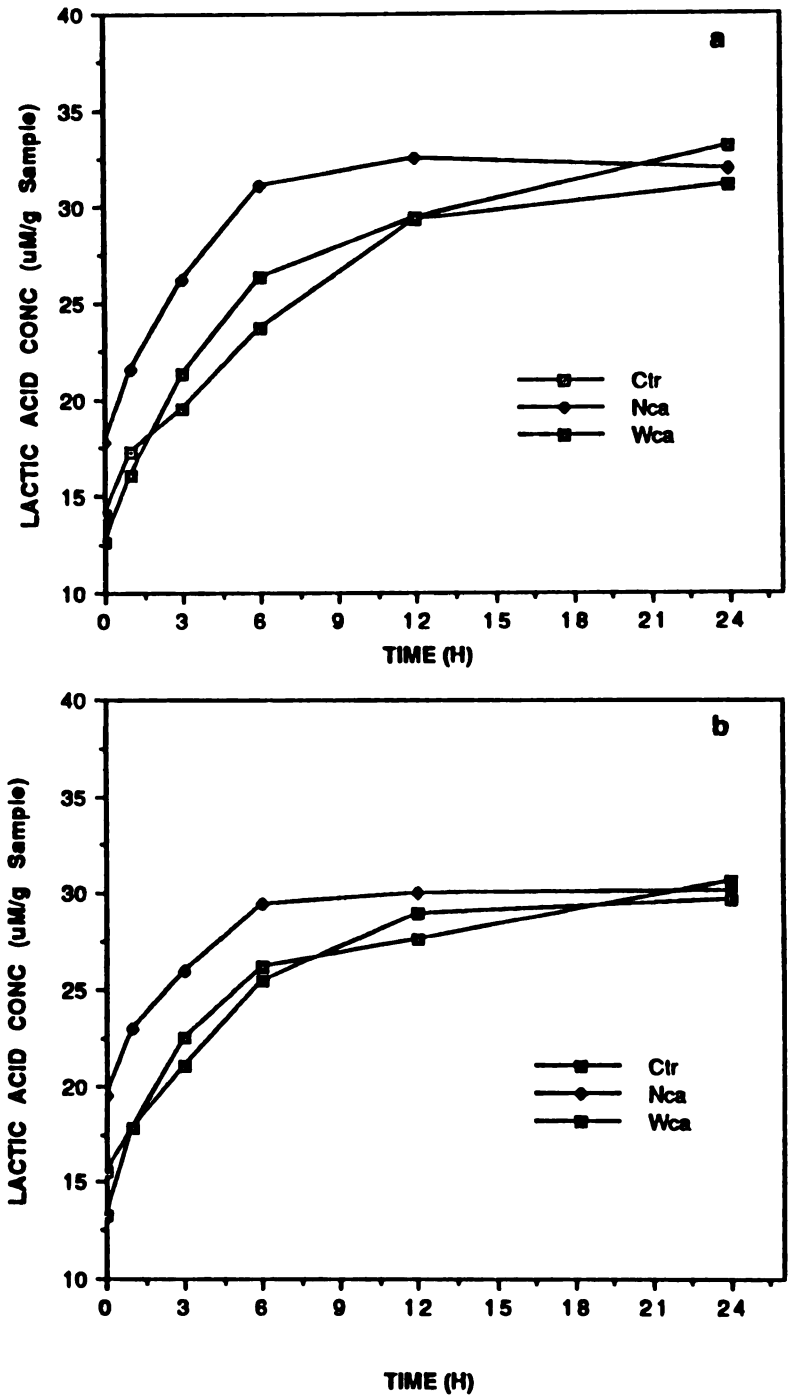


Fig 6a & b. Postmortem change in lactic acid concentration in ovine longissimus (a) and infraspinatus (b) muscles

TABLE 3.5. TREATMENT EFFECT ON THE DIFFERENCE IN LACTIC ACID CONCENTRATION WITHIN TIME*

Muscle	Time (h)	Contrast (non-orthogonal)*		
		Ctr vs NCa	Ctr vs WCa	NCa vs WCa
Longissimus	0	3.64	1.43	5.07
	1	4.26	1.2	5.46
	3	6.76	1.82	4.94
	6	7.39	2.61	4.78
	12	3.2	.16	3.04
	24	.86	1.99	1.13
Infraspinatus	0	4.02	2.2	6.22
	1	5.17	.01	5.16
	3	4.89	1.49	3.4
	6	3.88	.72	3.16
	12	1.05	1.32	2.37
	24	.44	.97	.53

* Absolute difference between the means of two treatments within a time. Values are obtained by calculating the difference between the values in Tables 3.1 and 3.2.

* Bonferoni t-test at 5% level of significance. LSD = 1.60 and 1.73 for longissimus and infraspinatus muscles, respectively.

Ctr = control; NCa = infused without $CaCl_2$, WCa = infused with $CaCl_2$.

TABLE 3.6. CHANGES IN LACTIC ACID CONCENTRATION WITH TIME WITHIN TREATMENTS*

Prime Cut	Contrast (non-orthogonal)*	Treatments		
		Ctr	NCa	WCa
Longissimus	0 h vs 1 h	3.16	3.78	3.39
	1 h vs 3 h	2.24	4.74	5.26
	3 h vs 6 h	4.24	4.87	5.03
	6 h vs 12 h	5.59	1.40	3.14
	12 h vs 24 h	1.77	.57	3.60
Infraspinatus	0 h vs 1 h	2.28	3.43	4.49
	1 h vs 3 h	3.27	2.99	4.75
	3 h vs 6 h	4.48	3.47	3.71
	6 h vs 12 h	3.48	.65	1.44
	12 h vs 24 h	.71	.10	3.00

* Absolute difference between the mens of two times within the same treatment. Values are obtained by calculating the difference between the values in Tables 3.1 and 3.2.

* Bonferoni t-test at 5% level of significance. The LSD for longissimus and infraspinatus muscles for comparison with table values are 1.60 and 1.73, respectively.

Ctr = control, NCa = infused without $CaCl_2$, WCa = infused with $CaCl_2$.

muscle. The result of the LAC change with time corroborated what we observed in the case of ATP and confirmed our conclusion that postmortem changes were completed by the first 6h in NCa.

Interrelationship Between pH, ATP and Lactic Acid

Bendall (1979) observed that a linear relationship exists between the relative pH, lactate and ATP concentration in different species of animals. In the present study, simple correlation coefficients (r) of -.98 and -.93 (pH vs LAC), -.96 and -.91 (ATP vs LAC) and +.96 and +.96 (pH vs ATP) were found for LD and IS muscles, respectively. A correlation coefficient of +.95 between temperature and pH, and temperature and ATP was noted. The high correlations observed between the various parameters determined in the present study are in agreement with similar findings previously published (Lundberg et al., 1987; Bendall, 1979; Winger et al., 1979; Tarrant and Mothersill, 1977; Bodwell et al., 1965). Because of the high correlations that between pH and the other products of glycolysis determined in the present study, we join other authors in suggesting the use of pH measurement alone to follow glycolytic changes in muscles postmortem (Lundberg et al., 1987; Pearson et al., 1973).

CONCLUSIONS

At the concentration used in this study, infusion of a blend of maltose, dextrose, polyphosphates, glycerin in ovine carcasses led to the acceleration of glycolysis, such that the process was completed within the first 6h PM. Infusion of the blend with $CaCl_2$ retarded glycolysis when compared to infusion without $CaCl_2$; nevertheless, glycolytic changes were faster in carcasses infused with $CaCl_2$ compared to controls. Glycolytic changes could be followed by simple measurement of pH postmortem. Further research is needed to ascertain the role each or a combination of ingredients play in postmortem glycolysis and how that would affect the quality of meat from the consumer and processing point of views.

CHAPTER 4

POSTEXSANGUINATION INFUSION OF OVINE CARCASSES: EFFECT ON TENDERNESS AND MUSCLE MICROSTRUCTURE

ABSTRACT

Twenty-four lambs were assigned to three treatment groups: 1) control (Ctr), 2) infused with 10% volume by weight of a tenderizing blend (NCa) and 3) NCa plus .015M $CaCl_2$ (WCa). Compared to Ctr and WCa, NCa treated samples had lower shear force values ($P<.05$) and higher percent change in myofibrillar fragmentation index ($P<.05$). SDS-PAGE of infused samples revealed the appearance at 24 h postmortem of 22-30 kd protein components. Scanning electron micrographs of NCa myofibrils showed that they were more fragmented, fractured, or split and have wider interfibrillar spaces compared to Ctr and WCa. The fracture plane of muscles immediately postmortem was along the endomysial-sarcolemmal sheath, while at 24 h postmortem the sheath was weakened enough for the fracture to occur along the surface of the myofibrils.

INTRODUCTION

The importance of tenderness in meat is underscored by the fact that it has been so extensively studied (Calkins and Seideman, 1988; Koohmaraie et al., 1988b; Cross, 1987; Lawrie, 1985; Karmas, 1970). Several theories have been advanced in an attempt to explain tenderness-toughness in meat. However, the most widely accepted views of tenderness-toughness are the ones that relate it to myofibrillar contraction and/or connective tissue characteristics (Bailey and Light, 1989; Stanley, 1983; Marsh, 1977). It is believed that postmortem conditions and treatment far outweigh live animal factors in determining tenderness in meat (Locker, 1985; Cross, 1979). Some of the postmortem conditions affecting tenderness include postmortem muscle pH and temperature (Dutson, 1983a; Marsh, 1983), muscle fiber contraction (Jaime, 1989; Bouton et al., 1973), rate of glycolysis (Smulders et al., 1990), muscle fiber fracture (Marsh et al., 1981), postmortem proteolysis (Asghar and Bhatti, 1987; Goll et al., 1983; Davey and Gilbert, 1973) and infusion of $CaCl_2$ (Koohmaraie et al., 1988b, 1989, 1990; Pearson et al., 1973). Tenderness has been assessed by shear force measurement (Shackleford et al., 1991), myofibrillar fragmentation index (Culler et al., 1978; Olson and Parrish, 1977) both transmission and scanning electron microscopy (Eino and Stanley, 1973; Cassens et al., 1963) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (Ducastaing et al., 1985; Yates et al.,1983). In the

studies described in chapters 1 and 2, the infusion of dextrose, maltose, glycerin and polyphosphates significantly improved tenderness in lambs and cows compared to control animals. The objectives of the present study were to further ascertain the impact of infusing these ingredients and $CaCl_2$ on tenderness as assessed by various methodologies and to relate the effect of the process to changes in the rate of glycolysis and to changes in muscle ultrastructure.

MATERIALS AND METHODS

Animals

The same animals used for the study described in chapter 1 and 3 were also used for this study.

Infusion and Tissue Sampling

Lambs were infused as described in chapter 1. Samples of infraspinatus, longissimus and semitendonosus muscles were taken at 0 and 24 h for scanning electron microscopy (SEM), and at the same periods in longissimus muscle for myofibrillar fragmentation index (MFI) measurement and for SDS polyacrylamide gel electrophoresis (SDS-PAGE). Loin chops were taken at 24 h for Warner-Bratzler shear force (WBS) and sarcomere length measurements.

Shear force (WBS) Determination

Shear force was determined on frozen and thawed (2 to 3°C overnight) lamb loin chops (2.2 cm thick). Chops were cooked in an convection oven (Honeywell Model 1860.2; Market Forge Co., Everett, MA) at 107°C oven temperature to an internal temperature of 71°C. Chops were cooled at 2-4°C for 24 h and 2 cores (1.27 cm diameter) were removed from each of three chops of each carcass. Each core was sheared three times (total of six readings per chop) with a Warner-Bratzler shear device.

Sarcomere length Determination

Sarcomere length was determined by measuring a total of

15 sarcomeres from each loin sample with a Wild microscope that had a phase contrast setting (Wild Heerbrugg, Switzerland) at a magnification of 400X by use of an eye piece micrometer standardized with a stage micrometer. The samples for sarcomere measurement were prepared as described by Smith et al. (1976).

Myofibrillar Fragmentation Index (MFI) Determination

MFIs were determined on fresh loin muscles according to the procedure of Olson et al. (1976) as modified by Culler et al. (1978), except EGTA (Sigma Chemical Company, St. Louis, MO) was used in the isolating medium instead of EDTA and absorbance (540nm) was read with a Spectronic 20 (Milton Roy Company, Rochester, NY) spectrophotometer.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

For SDS-PAGE, the isolation of myofibrils was done according to the procedure of Goll et al. (1974). One half mL of each sample of myofibrils isolated according to the procedure was dissolved in 2 mL of sample buffer (62.5% Tris-HCl, pH 6.8, 2% SDS, 5% MCE, 10% glycerol and 0.2% bromophenol blue). The samples were then heated in boiling water bath for 5 min and stored frozen at -30°C until used for electrophoresis. Electrophoresis was conducted on slab gels according to the procedure of Laemmli (1970) as modified by Babiker (1989), except 70 ug of protein were loaded to each 2 cm wide lane of the gel.

Scanning Electron Microscope Sample Preparation

Sample preparation for scanning electron microscopy (SEM) was a combination of the procedures described by Klomparens et al. (1986) and Cheng and Parrish (1976). Fresh longissimus, semitendinosus and infraspinatus muscle samples were placed in Petri dishes containing 2.5% gluteraldehyde (in 0.1M phosphate, pH 7.2) and diced into cubes 1mm X 1mm X 4mm with a sharp razor and fixed in 2.5% gluteraldehyde for one and half hour at room temperature. Samples were then washed twice in buffer (0.1M phosphate, pH 7.2) for 30 min each. Postfixation was carried out in 1% OsO_4 in 0.1M phosphate buffer at pH 7.2 for 2 hrs at room temperature. Samples were then washed and dehydrated in graded (25%, 50%, 75%, 95%, 100%) ethanol for 10 min each. Cryofracture was carried out by freezing in liquid nitrogen and fracturing with a razor blade. The fractured fragments were allowed to thaw in 100% ethanol. The samples were critical point dried using liquid carbon dioxide with a Balzer's critical point drier, and the dried fragments mounted on stubs with adhesive mounting tabs and then grounded with a conducting graphite paint. The samples were coated with gold using a sputter coater. Samples were examined and photographed in a JEOL JSM-35C scanning electron microscope using 10kV accelerating voltage, 15mm working distance and 250 um condenser lens aperture.

Statistical Analysis

The design employed for collection and analysis of samples for WBS, MFI and sarcomere length measurements was a complete randomized design (Gill, 1978a). One way ANOVA table was generated with a Microstat statistical package (Ecosoft, 1984). Comparisons (non-orthogonal) of the means was by Bonferoni t-statistics at 95% level of confidence.

RESULTS AND DISCUSSION

Table 4.1 compares the shear force values (WBS), myofibrillar fragmentation index (MFI) and sarcomere length (SL) of ovine longissimus muscle as a result of the various treatments employed in this study. Infused samples without $CaCl_2$ (NCa) had lower ($P<.05$) WBS and a higher ($P<.05$) percent increase in MFI over a period of 24 hrs compared to samples infused with $CaCl_2$ (WCa) and Controls (Ctr). No difference ($P>.05$) was observed between WCa and Ctr samples in the above measurements. A simple correlation coefficient, (r) of -0.5 was found between WBS and MFI. The greater tenderness (27%) observed in NCa samples compared to Ctr could be attributed to the faster rate of glycolytic changes (as was shown in chapter 3) in these samples as well as the combined effect of the ingredients infused into the carcasses. The importance of accelerating glycolysis for tenderness improvement has been demonstrated (Smulders et al., 1990; Ducastaing et al., 1985). The combination of high temperature and low pH might have favored some of the enzymes responsible for meat tenderization (Dutson, 1983a). The added phosphate could have contributed to the acceleration of glycolysis, solubilization of actomyosin, the chelation of divalent cations and consequently the increased tenderness (Lewis et al., 1986; Pearson and Tauber, 1984; Trout and Schmidt, 1983; Hamm, 1977). The other

Table 4.1. Effect of infusion on tenderness in ovine longissimus muscle.

Measure	Treatments			SEM
	Ctr	NCa	WCa	
Shear force (kg)	8.19[a]	5.96[b]	9.22[a]	.52
MFI (%) (520nm * 200)	64.71[a]	105.08[b]	51.37[a]	16.9
Sarcomere length (um)	1.82[a]	1.85[a]	1.63[a]	.07

Values are means of: 48 measurements, n = 8 for Shear force
16 measurements, n = 8 for MFI
150 sarcomeres, n = 8 for Sarcomere length.

MFI = Myofibrillar fragmentation index change over 24 hr period.
Any two means in the same row with the same superscripts are not significantly different ($P<.05$), by Bonferoni t-statistics.
Ctr = Control, NCa = No $CaCl_2$, WCa = with CaCl.
SEM = Standard error of the mean.

ingredients in addition to phosphate have been used in various combinations for tenderizing patents (Karmas, 1970). The disruption of muscle cells caused by the infusion process could lead to improved tenderness as a result of the process alone or in addition to the stimulation of glycolytic and proteolytic enzymes (Stanton and Light, 1990b; Hamm, 1982). As opposed to previously reported studies (Taylor and Etherington, 1991; Koohmaraie et al., 1988b, 1990, 1991; Alarcon-Rojo and Dransfield, 1989), infusion of $CaCl_2$ increased the toughness of the muscles by about 35% and 13% compared to NCa and Ctr treated samples, respectively. The lower concentration (.015M vs .3M) of $CaCl_2$ used in the present study could explain the difference observed. The infusion of $CaCl_2$ at concentration employed in this study results in calcium induced shortening (shorter SL, 12%) of the muscle fibers in WCA treated samples compared to NCa and Ctr (Pearson et al., 1973). The muscle fiber shortening could be the reason for the observed lower tenderness in WCa samples (Jaime et al., 1991; Smulders et al., 1990; Bouton et al., 1973).

The contention that the toughness of WCa samples was mainly because of muscle fiber shortening was supported by the result of the SDS-PAGE (Fig. 7). The appearance of 28, 29 and 30 kd protein components in the gels of the myofibrillar proteins of NCa and WCa at 24 hr postmortem indicate that proteolytic changes occurred faster in those samples compared

Fig. 7. Electrophoretic pattern of myofibrillar proteins from infused and control ovine longissimus muscles.

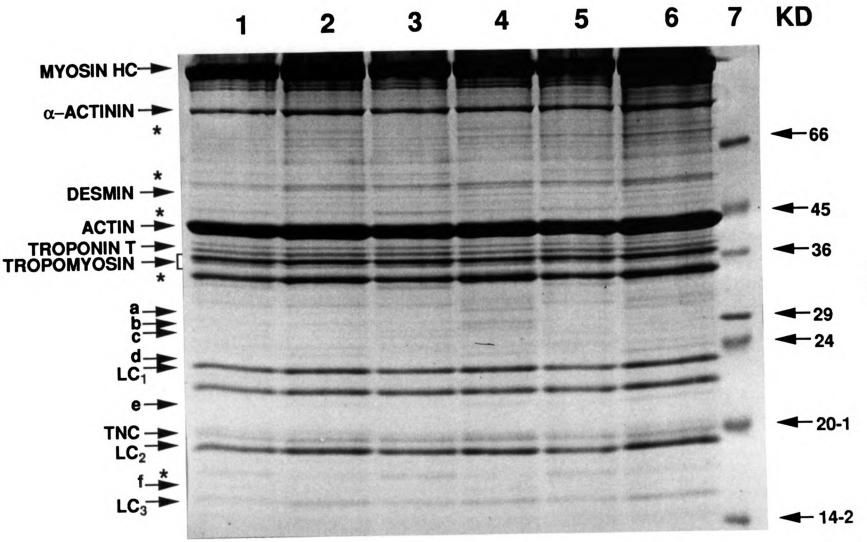
Lanes 1, 3 & 5 = Ctr, WCa & NCa samples at 0 h, respectively.

Lanes 2, 4 & 6 = Ctr, WCa & NCa samples at 24 h, respectively.

Lane 7 = Molecular weight markers

a, b, c, d & e = 30, 29, 28, 24 & 22 kd bands

* = Bands that changed in appearance or intensities at 24 h postmortem.


1
2
3
4
5
6
7
KD
MYOSIN HC
α–ACTININ
DESMIN
ACTIN
TROPONIN T
TROPOMYOSIN
a
b
c
d
LC1
e
TNC
LC2
f
LC3
66
45
36
29
24
20-1
14-2

to Ctr. Since the appearance of 30 kd is used as an index of myofibrillar proteolysis and tenderness and the fact that it is not affected by shortening (Babiker et al., 1989; Koohmaraie et al., 1984a and b; Yates et al., 1983; McBride and Parrish, 1977; Samejima and Wolfe, 1976), then the toughness of WCa samples could be attributed to shortening of fibers and not to the absence of proteolysis per se. This study demonstrated that it is possible to have a 30 kd protein in the gels of extracts from tough meat. Therefore, the appearance of 30 kd should only be used as an index of the rate of proteolysis but not of tenderness. Other bands observed in the gels of infused samples but not in the controls includes the appearance at 24 h of a 21-22 kd and 24 kd protein component. When gels prepared from 0 hr samples were compared to the 24 h ones, the following changes were also observed at 24 h: 1) the appearance of a protein band with a molecular weight > 66 kd, 2) an increase in the intensity of 55-56 kd protein, and 3) the complete disappearance of 34-35 kd and 17 kd protein bands. These observations were made in a total of 12 animals and are more clearly shown in the gels compared to the gel pictures. For that reason asterisks and letters of the alphabet were used to mark the bands of interest on the figure. Some of these changes in the protein bands could be indicators of the early steps in the process of tenderization. Previous studies (Whipple et al., 1990; Koohmaraie et al., 1988b, 1984b; Cheng and Parrish,

1978) did not show these changes, probably as a result of differences in the methods of gel preparations or because the quantity of proteins loaded (in relation to the width of the lanes of the gels) resulted in bands that are heavy enough to mask other bands. However, with the widely accepted view that the tenderizing process starts early postmortem (Mills et al., 1989; Koohmaraie et al., 1986; Zeece et al., 1986; Marsh, 1983), it is essential whenever SDS-PAGE is employed to ensure that the preparation allows the changes taking place within the first 24 h to be observed.

The scanning electron micrographs of the infraspinatus muscle (Fig. 8 A-F) serve as representative examples of several from which interpretations were made in the present experiment. Results indicate that when 0 h samples were fractured (Fig 8 A, C and E), they fractured along the endomysial-sarcolemmal sheath; whereas 24 h samples fractured along the surface of myofibrils. Subtle proteolytic changes in the endomysial-sarcolemmal tissues might have weakened the tissue resulting in the difference observed in the fracture planes (Jones et al., 1976; Eino and Stanley, 1973; Schaller and Powrie, 1971). The endomysium is the layer of connective tissue which lies immediately over the sarcolemma (basement membrane and plasmalemma) and the two tissues hardly ever separate under external pressure (Rowe, 1989; Orcutt et al., 1986). The endomysial complex is composed of a mixture of

Fig. 8A-F. Scanning electron micrographs of freeze fractured ovine infraspinatus muscle.

Fig A. Ctr muscle, 0 h PM. Note the presence of sarcolemmal sheath covering the myofibrills

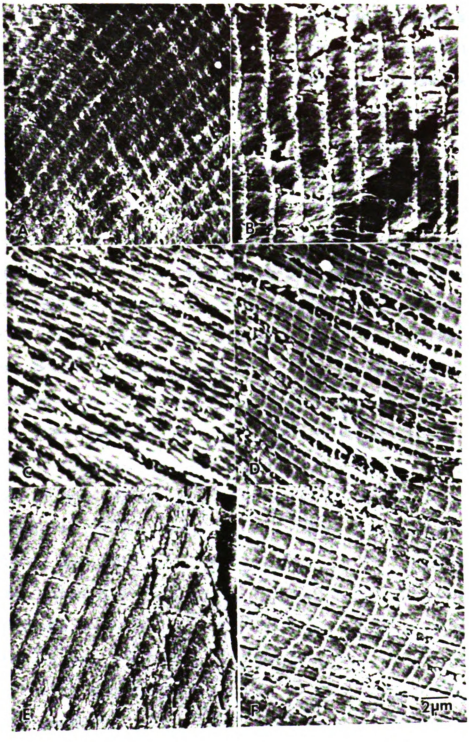
Fig B. Ctr muscle, 24 h PM. The sarcolemmal sheath visible in B is partially noticeable.

Fig C. NCa muscle, 0 h PM. The presence of sarcolemmal sheath is decernable.

Fig D. NCa muscle, 24 h PM. Note the amount of fragmentation of the myofibrills and the gab between the myofibrills.

Fig E. WCa muscle, 0 h PM. Presence of thick layer of sarcolemma can be seen. The folds on the micrograph could be as a result of muscle contraction.

Fig F. WCa muscle, 24 h PM. Myofibrillar fragmentation is less than in NCa at this h. The interfibrillar spaces are not as wide as those in NCa at the same time PM.


A
B
C
D
E
F
2μm

types I, III and V collagen (Bailey and Light, 1989). Recent studies indicated that proteolytic changes (though subtle) take place in the collagen component of the endomysial complex (Stanton and Light, 1990a; Bailey and Light, 1989; Mills et al., 1989). These changes might explain the differences observed in the fracture planes of 0 and 24 hr freeze fractured muscles. When the exposed myofibrillar surfaces were compared, the myofibrils of NCa (Fig. 8D) were more splintered and had wider interfibrillar spaces, compared to WCa (8F) and Ctr (8B). MFI was shown to account for more than 50% of the variations observed in aged bovine steaks (Culler et al., 1978; Olson and Parrish, 1977). A collagenalytic enzyme bound to skeletal muscle collagen was shown to cause splitting of collagen fibrils and presumably disintegration of muscle fibers related to tenderization (Suzuki et al., 1985). Although electron micrographs are difficult to interpret (Lewis et al., 1986; Cheng and Parrish, 1976), the changes observed in this study suggest that the interfibrillar distance, myofibrillar splitting and fracturing might be one way by which the process of tenderization takes place in conditioned muscles. In fact, very little fracturing of myofibrils was required to produce marked tenderization in electrically stimulated carcasses (Marsh et al., 1981).

In summary, the results of the present experiment demonstrate that at the concentration used in this study, a blend of maltose, dextrose, glycerin and polyphosphates could

be infused into carcasses to achieve marked improvement in tenderness within 24 hr postmortem. But infusion of the blend with $CaCl_2$ at the concentration used in this study resulted in the increased toughness of the meat. The appearance of, or changes in myofibrillar proteins with molecular weights > 66, 55-56, 34-35, 30, 29, 28, 24, 21-22 and 17 kd's in addition to the splitting, fracturing or differences in the relative distances between myofibrils, may help explain the process of tenderization. Further research is needed to ascertain the role of individual or combination of ingredients in the infusion process and to determine the effect of the ingredients on the activities of proteolytic enzymes.

SUMMARY AND CONCLUSIONS

The effects of the infusion of dextrose, maltose, glycerin, phosphate blend and $CaCl_2$ on tenderness, functional properties, postmortem metabolic changes and the ultrastructure of meat were investigated. Results of tenderness as determined by shear force measurement indicated that when compared to Ctr, infusion without Ca^{2+} improved tenderness significantly ($P< .05$) by 13% and 27% in beef and lamb loin muscles respectively. However, infusion with $CaCl_2$ caused increased toughness in lamb loins by about 35% and 13% compared to Nca and Ctr respectively. The increased toughness due to Wca resulted from calcium ion induced toughening of the muscles. The result of MFI change over 24 h corroborated what was observed with shear force measurement in lambs. The distribution of solution in the infused carcasses was not uniform. The amount of difference in MFF between controls and infused carcasses was in the order: chuck/shoulder > loin > round/leg. The amount of added moisture determined by MFF was found to be a better indicator of added moisture in whole carcass infusion compared to PFF and M:P. Results of Hunter L, a & b indicated that, in freshly cut lamb leg steak before storage, redness (a) in the samples was in the order: Nca > Ctr > Wca, but after storage (refrigerated and frozen) for 1 wk, 'a' value was in the order: Ctr > Nca > Wca. Lightness (L) and yellowness (b) was

increased in the steaks as a result of infusion. Frozen storage preserved the red color of meat but lowered its lightness and yellowness. However, the opposite effect on color was observed with refrigerated storage. Water-holding capacity (WHC) as determined by drip, thaw drip and cook losses was not affected by infusion in refrigerated samples, but WHC was lowered in frozen ones. Protein extractability was improved in beef due to infusion. Increased proteolytic activities were evidenced by the appearance of 22 to 30 KD protein components in the SDS-PAGE pattern of infused samples within 24 h PM. The rate of glycolysis was higher in infused samples compared to Ctr. The rate of glycolysis was in the order: Nca > Wca > Ctr. The scanning electron micrographs of freeze-fractured Nca samples shows evidence of wider inter-fibrillar spaces, more splitting and fracturing compared to Wca and Ctr.

The present study concluded that, bovine and ovine carcasses could be infused with 10% of their live body weight of a solution composed of dextrose, maltose, glycerin, and phosphates with the retention level of the solution differing in various parts of the carcass. Infusion of the ingredients at the concentration used in this study resulted in, improved tenderness, protein extractability, increased carcass dressed yields, faster rate of glycolysis and better color in freshly cut meats. Inclusion of $CaCl_2$ in the infusion solution at the concentration used in the present study could result in,

increased toughness, lower WHC and faster rate of deterioration in the color of meat. The process of tenderization may be more fully understood by following the appearance of or the changes in myofibrillar proteins with molecular weights 55-56, 34-35, 30, 29, 28, 24, 21-22 and 17. The understanding of the process could also be enhanced by such changes as the increase in the amount of splintering , fracturing and the relative distances between adjacent myofibrills in a muscle fiber. However, more studies are needed to, 1) further prove the observations made in this study, 2) determine the role of each ingredient and or combination of the ingredients on the various parameters measured in this study, 3) determine which groups of enzymes (CDPs and Cathepsins) play a major role in improving tenderness as a result of the infusion process, 4) investigate the effects of the ingredients particularly $CaCl_2$ on glycolytic enzymes and the enzymes responsible for changes in color, 5) examine the feasibility of using the infusion process to fortify meats, 6) explore the economics of applying the process to the red meat industry, 7) conduct sensory panels on tenderness and taste and 8) conduct some microbiological studies of the process.

APPENDICES

APPENDIX

TABLE 1. EFFECT OF INFUSION ON POSTMORTEM CHANGES IN OVINE LONGISSIMUS MUSCLE*

Treatment	Time (h)	Temp(°C)	pH	ATP	Lactic Acid
Ctr	0	39.68±1.1	6.96±.11	2.51±.9	14.97±1.6
	1	35.45±.7	6.88±.1	2.38±.7	17.23±1.5
	3	23.14±1.1	6.75±.13	2.37±.6	19.47±1.5
	6	13.22±.8	6.46±.28	1.73±.8	23.71±.5
	12	5.01±.4	6.05±.2	.19±.1	29.30±1.4
	24	3.13±.1	5.72±.20	0	31.07±2.3
NCa	0	36.58±.62	6.74±.1	2.04±.8	17.71±2.1
	1	31.85±1.1	6.66±.13	2.18±.5	21.49±1.8
	3	21.67±1.1	6.25±.06	1.63±.7	26.23±1.7
	6	13.09±.89	5.85±.13	.37±.3	31.10±1.7
	12	5.17±.36	5.63±.14	0	32.50±1.4
	24	3.13±.11	5.58±.15	0	31.93±1.2
WCa	0	37.15±.66	6.99±.14	2.70±1	12.64±2.5
	1	33.14±.9	6.88±.15	2.78±.8	16.03±3.4
	3	22.10±1.2	6.52±.26	2.45±.8	21.29±3.6
	6	12.78±1.7	6.18±.29	1.23±.6	26.32±2.2
	12	5.05±.22	5.84±.24	0.03±.07	29.46±3.0
	24	2.94±.1	5.66±.15	0	33.06±2.1

* All values are average observations of 8 carcasses, and ATP and Lactic acid are expressed as μmols/g sample.

Ctr = control, NCa = infused without $CaCl_2$, WCa = infused with $CaCl_2$

APPENDIX

TABLE 2. EFFECT OF INFUSION ON POSTMORTEM CHANGES IN OVINE INFRASPINATUS MUSCLE*

Treatment	Time (h)	pH	ATP	Lactic Acid
Ctr	0	7.06±.17	2.00±.7	15.49±2.8
	1	6.83±.14	2.44±.8	17.77±2.5
	3	6.66±.07	1.62±.5	21.04±2
	6	6.41±.1	.81±.5	25.52±3.2
	12	6.12±.15	.10±.1	29.00±3.1
	24	5.94±.19	0	29.71±3.2
NCa	0	6.77±.1	1.94±.8	19.51±2.7
	1	6.60±.14	1.80±.8	22.94±2.3
	3	6.27±.04	1.00±.6	25.93±2.3
	6	5.97±.16	.24±.3	29.40±3.2
	12	5.80±.13	0	30.05±2.8
	24	5.77±.22	0	30.15±2.5
WCa	0	7.02±.18	2.26±.8	13.29±1.8
	1	6.80±.18	1.94±.7	17.78±2.3
	3	6.49±.17	1.62±.5	22.53±1.8
	6	6.14±.15	.60±.3	26.24±1.9
	12	5.95±.17	.10±.1	27.68±2.4
	24	5.82±.23	0	30.68±4.1

*All values are average of observations of 8 carcasses, and ATP and Lactic acid are expressed as μmols/g sample.

Ctr = control, NCa = infused without $CaCl_2$, WCa = infused with $CaCl_2$

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