ERIZATION, CALPAIN/CALPASTATIN ACTIVITIES AND OSMOLALITY OF 6 DIFFERENT BEEF MUSCLES

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MARY: Of a total of 4 Friesian–pie noire cows, *Mm. diaphragma pedialis, supraspinatus, rectus abdominis, triceps brachii, longissimus semimembranosus* were sampled at different times *post mortem*. Tenderness, calpain and calpastatin activities, osmolality and sarcomere were assessed. A strong correlation (r=0.93) was found between tenderization and the ratio of calpain I and calpastatin of different Osmolality of the different muscles was significantly correlated (r=–0.73) to tenderness at 8 days *p.m.*

DUCTION: It is well known that meat tenderness increases gradually during *post mortem* storage of meat. As reviewed by OUALI 1991), tenderization is a variable process depending on a number of biological factors like age, sex, muscle type and animal species. In growth promotors may affect tenderization, as can the application of technologies like electrical stimulation and rapid cooling. ing the variability in the speed of tenderization between different muscles, three major points of interest can be distinguished (OUALI, (1) levels of proteases and inhibitors, (2) sensitivity of muscle proteins to proteolysis and (c) osmotic pressure.

During the last decade the evidence has accumulated that the calcium dependent protease calpain I (μ–calpain) and its endogenous r, calpastatin, play a central role in tenderization. Differences in tenderization between species could not be explained by differences in activity (ETHERINGTON *et al.*, 1987). This result was confirmed by OUALI and TALMANT (1990), but these investigators also ured calpastatin activities and found large differences in muscle calpastatin content between species and muscles. It was concluded that the f calpain and calpastatin can explain differences in the speed of tenderization. This conclusion was confirmed by results on differences in ness and calpastatin content between species (KOOHMARAIE *et al.*, 1991a) and bovine breeds (WHEELER *et al.*, 1990; WHIPPLE *et* 90); SHACKELFORD *et al.*, 1991). Furthermore, toughening of meat from different species as a result of β–agonist administration to e lean deposition could be explained by a decrease in calpain I activities and/or an increase in calpastatin activities (BEERMANN *et al.*, KOOHMARAIE and SHACKELFORD, 1991; KOOHMARAIE *et al.*, 1991b).

The pH decline in *pre–rigor* muscles coincides with an increase in osmotic pressure, i.e. ionic strength. The osmotic pressure in igor muscles is muscle type dependent and increases with the contraction speed of the muscles. This means that the highest osmolality is nd in fast–twitch muscles, which also show the fastest alterations of the myofibrillar structure *post mortem*. As reviewed by MONIN and 1 (1991), *post mortem* changes in osmotic pressure might affect meat tenderness in different ways. The ionic strength in *post mortem* ay is sufficient to dissociate contractile proteins and consequently alter the integrity of myofibrils. As a consequence the hydrolytic action dogenous proteases might be facilitated. Furthermore, differences in ionic strength might affect tenderness influencing the waterholding ity of muscles through a possible effect on the degree of shrinking or swelling of the myofibrillar lattice.

The results presented here are part of an investigation to determine the relationship between different biochemical and biophysical eristics of muscles and the rate and extent of tenderization. A full report on this study will be presented after collection of all data and ete statistical analysis.

RIALS AND METHODS: Four Friesian/pie noire cows (about 6 years of age) were obtained from commercial markets. After e–bolt stunning, exsanguination and dressing, *Mm. diaphragma pedialis* (DI), *supraspinatus* (SS), *rectus abdominis* (RA), *triceps* 1, *longissimus* (LO) and *semimembranosus* (SM) were excised within half an hour *post mortem*. Muscles were divided into five parts, ere packaged and kept at 15°C during the first 19 hours *post mortem*. Subsequently the muscles were stored in a cold room (0–2°C) up to 8 *post mortem*.

Rheological measurements: The rheological properties of the myofibrillar structure were measured according to LEPETIT and SALE 0), using a sinusoidal compressive test with a 20% compression ratio. Measurements were performed at 1, 2 and 8 days *post mortem*.

2 - Sarcomere length: Sarcomere length was determined according to Cross *et al.*. (1980–81).

3 - Separation of calpains and calpastatin and activity measurements: Five g of muscle was homogenized in 8 volumes of (50 mM Tris/HCl, 3 mM EDTA, 10 mM MCE, 150 nM pepstatin A, pH 8.3, 4°C) using a Polytron homogenizer. The homogenate was ke ice for 15 min and centrifuged (20000 g, 15 min, 2°C). The supernatant was filtrated over glasswool, adjusted to pH 7.5 with HCl and NaCl was added to a concentration of 0.5 M. Fifteen ml of the extract was loaded on a phenylsepharose CL–4B column (1*4 cm, 1 ml Pharmacia), previously equilibrated with buffer A (20 mM Tris/HCl, 0.4 mM EDTA, 0.5 M NaCl, 10 mM MCE, pH 7.5). The column washed with 10 ml of buffer A. The unbound fraction contained calpastatin. Calpains were eluted with 10 ml buffer B (20 mM Tris/HCl mM EDTA, 10 mM MCE, pH 7.5). Eight ml of the calpains–containing eluate was loaded on a mono–Q (HR 10*10, Pharmacia), previo equilibrated with buffer B. Calpain I and II were separated with a gradient of 0 to 0.5 M NaCl using buffers A and B. Calpain activity determined using casein as substrate. 0.5 ml of the calpain containing fractions were incubated with 0.5 ml incubation medium (10 m casein, 100 mM Tris/HCl, 10 mM MCE, 10 mM $CaCl_2$, pH 7.5). After 20 min incubation at 30°C the reaction was stopped by addition o ml TCA (10% w/v). TCA was added immediately to the blanks. The precipitate was spun down and the increase in TCA–soluble peptides measured at 278 nm. One unit of calpain activity was defined as an increase in absorption of 0.001/min/g muscle. Calpastatin activity determined by measuring the decrease in calpain II activity when incubated with calpastatin containing eluent. The calpastatin containing fra was heated during 3 min at 100°C, centrifuged and the supernatant was diluted with different amounts of buffer A. Of a calpain II stock sol 0.5 ml was incubated with 0.5 ml of different dilutions of the calpastatin–containing fraction and 0.5 ml of incubation medium. After 20 incubation at 30°C the reaction was stopped by addition of 0.5 ml TCA (10% w/v). TCA was added immediately to the blanks. Calpas activity was calculated from the slope of the linear part of the inhibition curve. One unit of calpastatin activity was defined as the amount w inhibited one unit of calpain II activity.

4 - Osmolality: Osmolality measurements were performed according to Bonnet *et al.* (1992).

RESULTS AND DISCUSSION:

The results of the rheological measurements are given in Figure 1. It is essential to realize that only the myofibrillar component of tender was measured with the used method. Thus, differences in tenderness cannot be explained by differences in the amount and quality of connec tissue. A considerable difference was observed in the speed of tenderization between muscles. SM and LO show the fastest tenderization RA the slowest. Differences in tenderness could not be explained by differences in sarcomere length. The respective sarcomere lenghts o different muscles were: 1.85±0.12 (DI), 1.94±0.09 (SS), 1.95±0.12 (RA), 2.03±0.15 (TB), 1.92±0.09 (LO) and 1.83±0.05 (SM).

The results of the calpain I and calpastatin measurements are given in Figures 2 and 3. Calpain II activity remained rather cons throughout the ageing period. The respective calpain II activities (1 h *p.m.*) were: 135±13 (DI), 120±24 (SS), 138±13 (RA), 114±17 (119±11 (LD) and 110±14 (SM). The initial calpain I activity varied little between muscles, and cannot explain the observed differences in speed of tenderization. When calpain I activity decline during the ageing period is compared with the rheological measurements a similar pat can be observed. It is tempting to explain this parallel with the theory that calpains, once activated, gradually lose their activity throug autolytic process. This would mean that a loss in calpain activity would indicate that the enzyme has been active and possibly cau tenderization. However, the time scales of tenderization and calpain I activity decline are different. At 2 days *p.m.* the greatest decline in cal I activity has already taken place while the largest part of the tenderization response takes place between 2 and 8 days *p.m.*

Regarding the calpain/calpastatin system it is clear that the largest differences between muscles are found in the amount of calpast (Fig 3). When the calpastatin content of the different muscles is compared with the tenderization response a striking similarity is observed. T muscles with the lowest calpastatin activity (LO and SM) show the fastest tenderization, whereas RA shows both the slowest tenderization a the highest calpastatin content. Furthermore, the speed of calpain I activity decline parallels the calpastatin distribution over the differe muscles. This indicates that the amount of calpastatin determines the speed of tenderization through regulation of calpain I activity. In this respe the ratio of calpain I and calpastatin could be considered as an indicator for the activity of calpain I, and possibly the rate of tenderizatio Indeed, a strong correlation (r=0.93) was found between the relative tenderization between 2 and 8 days *p.m.* and the calpain I/calpastatin ra (2 days *p.m.*) of different muscles (Fig 4). When the same concept was applied to each indivual muscle a much weaker correlation was found (r=0.44), though still significant ($p<0.05$).

A possible explanation for the difference in the time scale between calpain I activity decline and tenderization could be that proteolysis of illar proteins is only the first step in the tenderization process. A possible second step could be further disintegration of the myofibrillar depending on the osmotic pressure attained in *post–rigor* muscle. A significant correlation (r=–0.73; p<0.05) was found between ity of the muscle juice and tenderness at 8 days p.m. (Fig 5). When the same comparison was made for each individual muscle a but still significant correlation was found (r=–0.58, p<0.05).

Our results support the hypothesis of OUALI (1990) that tenderization is dependent on both enzymatic and physical disintegration of the illar structure. In agreement with earlier observations, differences in the speed of tenderization could not be explained by differences in l activity alone. The ratio of calpain I and calpastatin seems to be more important in this respect. However, it is not clear how calpastatin bit calpains at the pH of *post–rigor* muscles. Calpastatin gradually loses its inhibiting capacity *in vitro* when the pH is lowered from 7.5 d below pH 6.0 no inhibition takes place (GEESINK *et al.*, unpublished results). Possibly, the relation between pH and inhibition of by calpastatin is different *in situ*.

LUSIONS:

ent results confirm that muscle type is an important factor in meat tenderization. Possible muscle type dependent determinants of meat ation are the ratio between calpain I and calpastatin, and the osmotic pressure attained in *post–rigor* muscles.

RENCES:

BEERMANN, D.H., S.Y. WANG, G. ARMBRUSTER, H.W. DICKSON, E.L. RICKES and J.G. LARSON. 1989. Influences of b-L-655,871 and electrical stimulation on post-mortem muscle metabolism and tenderness in lambs. Recip. Meat Conf. Proc. Am. Meat oc. 42:54.

BONNET, M., A. OUALI and J. KOPP. 1992. Muscle osmotic pressure as assessed by differential scanning calorimetry (DSC). Int. J. d. Technol. (in press).

CROSS, H.R., R.L. WEST and T.R. DUTSON. 1980–81. Comparison of methods for measuring sarcomere length in beef rofinosus muscle. Meat Sci. 5:261.

ETHERINGTON, D.J., M.A.J. TAYLOR and E. DRANSFIELD. 1987. Conditioning of meat of different species. Relationship n tenderizing and the levels of cathepsin B, Cathepsin L, calpain I, calpain II and α-glucuronidase. Meat Sci. 20:1.

KOOHMARAIE, M. and S.D. SHACKELFORD. 1991. Effect of calcium chloride infusion on the tenderness of lambs fed a β-rgic agonist. J. Anim. Sci. 69:2463.

KOOHMARAIE, M., G. WHIPPLE, D.H. KRETCHMAR, J.D. CROUSE and H.J. MERSMANN. 1991a. Postmortem proteolysis in rnus muscle from beef, lamb and pork carcasses. J. Anim. Sci. 69:617.

KOOHMARAIE, M., S.D. SHACKELFORD, N.E. MUGGLI–COCKETT and R.T. STONE. 1991b. Effect of the β-adrenergic L-644,969 on muscle growth, endogenous proteinase activities, and postmortem proteolysis in wether lambs. J. Anim. Sci. 69:4823.

LEPETIT, J. and P. SALE. 1985. Analysis of the rheological behavior of meat by a sinusoidal compressive device. Sci. Alim. 5:521.

MONIN, G. and A. OUALI. 1992. Muscle differentiation and meat quality. In: R. Lawrie (Ed.). Developments in Meat Science 5. p.89.

OUALI, A. 1990. Meat tenderization: possible causes and mechanisms. A review. J. Muscle Foods 1:129.

OUALI, A. and A. TALMANT. 1990. Calpains and calpastatin distribution in bovine, porcine and ovine skeletal muscles. Meat Sci.

OUALI, A. 1991. Sensory quality of meat as affected by muscle biochemistry and modern technologies. In: L.O. Fiems, B.G. Cottyn l. Demeyer (Eds.). Animal Biotechnology and the Quality of Meat Production. Elsevier Sci. Publ. B.V., Amsterdam. p. 85.

SHACKELFORD, S.D., M. KOOHMARAIE, M.F. MILLER, J.D. CROUSE and J.O. REAGAN. 1990. An evaluation of tenderness longissimus muscle of angus by hereford versus brahman crossbred heifers. J. Anim. Sci. 69:171.

WHEELER, T.L., J.W. SAVELL, H.R. CROSS., LUNT, D.K. and S.B. SMITH. 1990. Mechanisms associated with the variation in ness of meat fron brahman and hereford cattle. J. Anim. Sci. 68:4206.

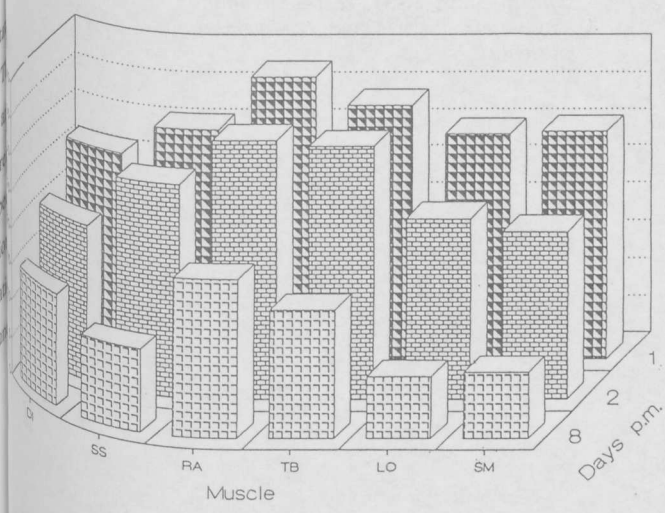

Figure 1. Tenderness (myofibrillar component; N/cm^2) of *Mm. diaphragma pedialis* (DI), *supraspinatus* (SS), *rectus abdominis* (RA), *triceps brachii* (TB), *longissimus* (LO) and *semimembranosus* (SM) at 1, 2 and 8 days *post mortem*.

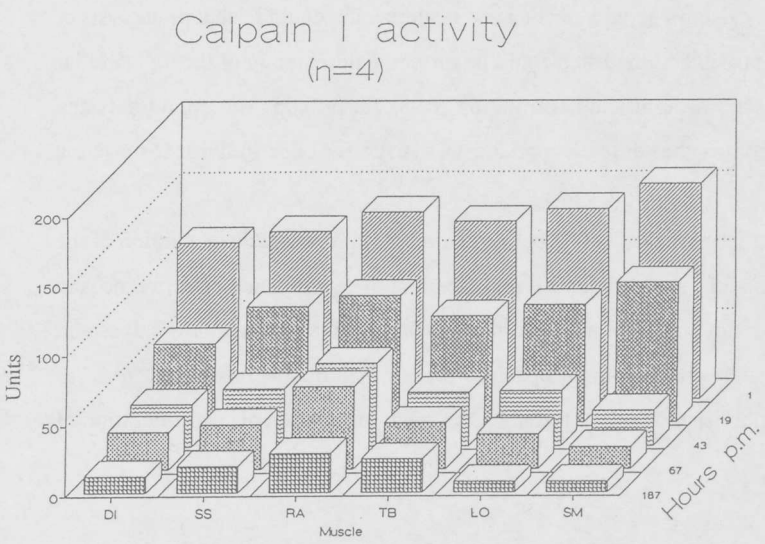

Figure 2. Calpain I activity (ΔA_{278}/min/g*1000) of *Mm. diaphragma pedialis* (DI), *supraspinatus* (SS), *rectus abdominis* (RA), *triceps brachii* (TB), *longissimus* (LO) and *semimembranosus* (SM) at 1, 19, 43, 67 and 187 hours *post mortem*.

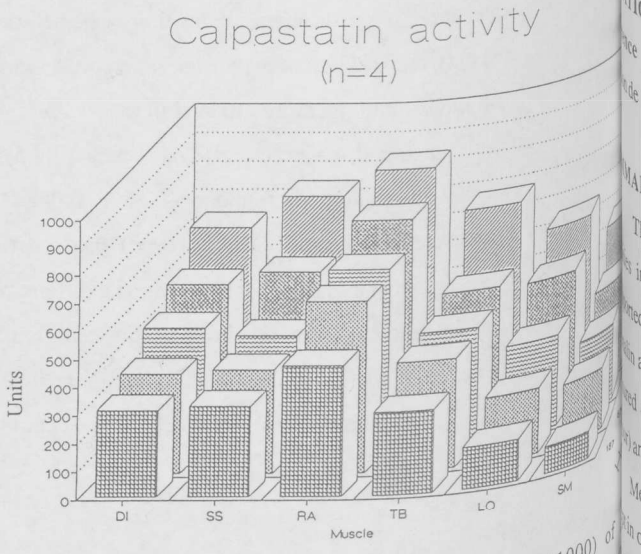

Figure 3. Calpastatin activity (-ΔA_{278}/min/g*1000) of *diaphragma pedialis* (DI), *supraspinatus* (SS), *rectus abdominis* (RA), *triceps brachii* (TB), *longissimus* (LO) and *semimembranosus* (SM) at 1, 19, 43, 67 and 187 hours *post mortem*.

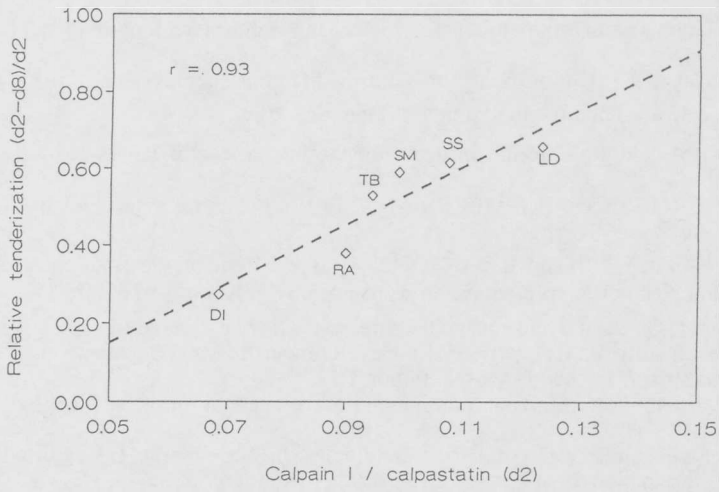

Figure 4. The relation between the ratio of calpain I and calpastatin activities at 2 days *post mortem* and the relative tenderization of *Mm. diaphragma pedialis* (DI), *supraspinatus* (SS), *rectus abdominis* (RA), *triceps brachii* (TB), *longissimus* (LO) and *semimembranosus* (SM) between 2 and 8 days *post mortem*.

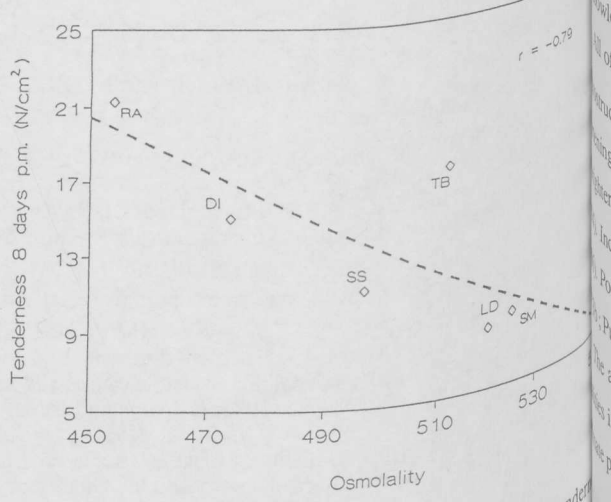

Figure 5. The relation between the osmolality and tender (myofibrillar component; N/cm^2) of *Mm. diaphragma pedialis* *supraspinatus* (SS), *rectus abdominis* (RA), *triceps brachii* *longissimus* (LO) and *semimembranosus* (SM) at 8 days *mortem*.