ACTIVITIES OF CALPAIN, CALPASTATIN, CATHEPSIN B+L AND CYSTATIN IN DANISH LANDRACE PIGS *ANNO* 1976 AND 1995

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Background
A group of Danish Landrace pigs was maintained without selection since 1976. Carcass lean content, daily gain and feed conversion differ significantly in pigs sampled from Danish Landrace representing *anno* 1976 and 1995 (Petersen *et al.* 1996). The rates of *in vivo* protein turnover in these two groups are therefore likely to differ. Altered rates of protein turnover could be related to different *in vivo* levels of proteolytic enzymes and these in turn could participate in post-mortem meat tenderisation.

Objectives
The purpose of this study was to investigate activities of proteolytic enzymes and their inhibitors in samples from two groups of Danish Landrace pigs with different growth rates and feed conversion efficiencies.

Methods
The investigation comprised a group of 40 Danish Landrace pigs *anno* 1976 and 37 pigs *anno* 1995. Samples for enzyme measurements were taken from *longissimus dorsi* at 42 min, 24 h or 6 days post-mortem, frozen in liquid nitrogen and stored at -80 °C until further analysis. Samples for shear force, cooking loss and thaw loss were frozen at 24 h post-mortem and samples for taste panel assessment at 4 days post-mortem and stored at -20 °C until analysis. Warner-Bratzler shear force and cooking loss was measured according to Møller (1981) using a heat treatment of 80 °C for 60 min. *μ-Calpain, m-calpain and calpastatin* were measured after separation of the proteins using an FPLC procedure (Iversen *et al.*, 1993). *Cathepsin B+L* was measured in a homogenate (total cathepsin B+L) and in the supernatant after centrifugation 100.000 x *g* for 60 min (free cathepsin B+L) as described by Ertbjerg *et al.*, 1994. *Cystatin* was measured in the soluble fraction. The method was based on the ability of cystatin to inhibit papain activity and were performed after modification of a described method (Katunuma and Kominami, 1995). SDS-PAGE were performed on 8-16% gradient gels and evaluated by densitometric scans.

Results and discussion
Samples from Danish Landrace pigs *anno* 1976 showed a tendency to decreased thaw loss and significantly ($P < 0.05$) decreased cooking loss as compared to samples from pigs *anno* 1995 (Table 1). Apparently, these results cannot be explained on basis of pH, since no differences in pH at 45 min or 24 h post-mortem between *anno* 1976 and 1995 were found (Petersen *et al.* 1996). However, meat from *anno* 1995 appeared more light in colour (Petersen *et al.* 1996; Juncher *et al.* 1996). Unaged samples from the two groups of pigs showed no differences in maximum shear force, although a tendency to increased final yield in *anno* 1976 were observed (Table 1). Final yield has been suggested to be related to the collagenous component of meat toughness (Møller, 1981), which has been reported to increase with animal age. Increased final yield of *anno* 1976 would thus be expected as these pigs took 26 days longer to reach the slaughter weight of 100 kg (Petersen *et al.* 1996). After ageing 4 days at 2 °C the *anno* 1976 group was ranked as more tender by taste panel assessment (Tabel 1).

At 42 min post-mortem the activities of the calpain system were measured. No significant differences were observed between the two groups of pigs for μ-calpain, m-calpain or calpastatin (Table 2). Alterations in growth performance traits of the pigs seemingly did not result in changes in the calpain system. The proteolytic potential of the calpain system would thus at time of slaughter be expected to be identical in pigs *anno* 1995 and 1976.

Table 1. Thaw loss, Cooking loss and Warner-Bratzler shear force 24 h post-mortem and taste panel assessed tenderness 4 days post-mortem in Danish Landrace pigs *anno* 1995 (n=34) and 1976 (n=37).

Anno	Thaw loss (%)	Cooking loss (%)	Max Shear Force (N/cm²)	Final Yield (N/cm²)	Tenderness[c]
1995	7.89	29.1[a]	50.5	15.7	9.9[a]
1976	7.25	27.1[b]	49.8	18.1	11.3[b]

[a,b]Means in the same column with different superscripts differ significantly ($P < 0.05$).
[c]Taste panel assessment at 4 days post-mortem using fifteen point rating scales (0 to 15) with higher numbers indicating increasing tenderness.

Table 2. Calpain and calpastatin activity (U/g muscle ± s.d.) 42 min pm in Danish Landrace pigs *anno* 1995 (n=5) and 1976 (n=5).

Anno	μ-Calpain	m-Calpain	Calpastatin
1995	0.14 ± 0.07	0.68 ± 0.08	0.74 ± 0.35
1976	0.15 ± 0.06	0.62 ± 0.08	0.72 ± 0.18

Table 3 shows that samples from the 1995 group exhibited increased total and free cathepsin B + L activities ($P < 0.05$ and 0.1 for means averaged across time of total and free cathpesin B + L, respectively). The group of pigs having highest daily gain and feed conversion thus also showed highest post-mortem activities of cathepsin B + L. Although samples had been frozen, results showed a tendency to increased free cathepsin B + L activity with increasing storage time and thus indicate a gradual release of lysosomal enzymes with time post-mortem. Since freezing is known to destabilize the lysosomal membrane, lower initial level of free cathepsin B + L and a more pronounced effect of storage time would have been expected if the measurements had been performed on unfrozen samples.

Cystatins are a special group of proteins of low and high molecular weight that inhibit some cysteine proteinases such as cathepsins and papain. In the soluble fraction there were no significant differences of cystatin activities between samples from pigs *anno* 1995 and 1976 (Table 3). Furthermore, results did not indicate that cystatins were degraded or otherwise became less active during storage. The difference in measured cathepsin B + L activities between the two groups of pigs and the increased free activity with storage time therefore seems to reflect differences of the level of cathepsin B and L rather than changes in cystatin.

Table 3. Cathepsin B+L and cystatin activity (mU/g muscle ± s.d.) and density of 31 kDa protein band (% of all proteins in SDS-PAGE lane) in Danish Landrace pigs *anno* 1995 (n=9) and 1976 (n=9).

Trait	*Anno*	42 min pm	1 day pm	6 days pm	Average
Total cathepsin B+L	1995	4.14	3.81	3.74	3.90[a]
	1976	3.51	2.90	2.76	3.06[b]
Free cathepsin B+L	1995	3.05	3.13	4.03	3.41
	1976	2.66	2.55	3.16	2.79
Cystatin	1995	9.6	10.6	10.2	10.1
	1976	9.4	10.7	11.5	10.5
31 kDa band	1995	0.30	0.46	0.49	0.42[a]
	1976	0.66	0.64	1.22	0.84[b]

[a,b]Within trait means in the same column with different superscripts differ significantly ($P < 0.05$).

SDS-PAGE showed that the density of the 31 kDa band increased from 42 min to 6 days (Table 3). The density of the appearing fragment was approximately 100% higher in samples from pigs *anno* 1976. The corresponding band in bovine samples has been identified as a degradation product from troponin T, and has been suggested to be an indicator of post-mortem proteolytic activity. It is therefore likely that samples from the *anno* 1976 group had an increased post-mortem proteolysis as compared to samples from *anno* 1995, which is also in accordance with the observed increase in tenderness after ageing. However, this altered post-mortem proteolysis could not be explained on basis of the measured activities of neither the calpain (Table 2) nor the cathepsin (Table 3) proteolytic systems.

Conclusions

Dansih Landrace pigs maintained without selection since 1976 was compared to Landrace pigs *anno* 1995. At 42 min post-mortem no significant differences between pigs *anno* 1976 and 1995 in the activities of m-calpain, μ-calpain and calpastatin were seen. Cathepsin B + L activities were measured 42 min, 1 day or 6 days post-mortem. Significantly higher total and free cathepsin B + L were observed in samples from pigs *anno* 1995. Results showed no difference between pigs in average cystatin activities. SDS-PAGE showed higher intensity of 31 kDa band in samples from pigs *anno* 1976. Overall, *anno* 1995 pigs having higher *in vivo* feed conversion also showed increased post-mortem cathepsin B + L activities, but decreased post-mortem proteolysis as evaluated by SDS-PAGE and panel assessed tenderness after ageing.

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