PE1.37 Changes in shear force of meat by age in pigs: The role of the calpain system at gene and activity level 245.00

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***Abstract*—We here report the results on gene expression (mRNA) of the calpains and calpastatin, activity of µM-calpain and calpastatin, and shear force in pig longissimus at various ages. From other studies it is known that the muscle protein degradation rate decreases by age. Because protein degradation rate is regulated by the net calpain activity initiating the myofibrillar disassembly and also determine the rate of post mortem tenderization of meat, we hypothesize that the shear force may increase by age in pigs. The present experiment was carried out on 14 litters of the D(LY) breed each of 3 castrated male pigs and 3 female pigs. The pigs were slaughtered at various ages: d28, d70, d90, d140, d161, and d182. The pigs were penned individually and fed standard diets for the grower and finisher period. From d28 to d140 gene expression of muscle calpains and calpastatin declined by increasing age. From d140 to d182 of age gene expression of calpains remained almost unchanged. However, the gene expression of calpastatin continued to decrease until d182 of age. The activity of µM-calpain decreased from d28 to d90, but returned to almost initial values from d140 to d182 of age. In contrast, the inhibitory activity of calpastatin was unaltered from d28 to d140, but decreased thereafter until d182. The shear force decreased from d28 to d140 and remained unchanged up to d182 if age. These data suggests that proteolysis is changed initially due to a decrease in the net activity of µM-calpain. In contrast to our hypothesize, shear force was highest in younger animal and lower in older pigs. We have no explanation for that, but other factors like the intra-muscular fat, collagen content and solubility, and sacomere length may contribute to the development of the shear force by age.**

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***Index Terms*—Age, Pigs, Calpains, Shear Force**

I. Introduction

The weight of slaughter pigs is gradually increasing under Danish conditions and since the nineties and till now the slaughter weight has increased from approximately 95 to 110 kg body weight. Furthermore, during the summer time there also is a market for small pig carcasses like veal meat. Thus, in order to optimise the meat quality, and especially the tenderness, there is an interest in elucidating the age dependent development of tenderness.

Results have shown that the fractional rate of protein synthesis (g muscle protein synthesised /100 g protein/per day, %/day; FRS) decreases curvilinear from a high value in new born pigs to a low value in 100 kg pigs [1, 2]. Similar changes but at a lower level occur in the rate of muscle protein degradation (FRD). The difference in FRS and FRD, termed the fractional rate of gain (FRG), consequently decreases following a similar pattern as do the FSR and FRD. The alteration in FRD by age may be due to a corresponding decrease in the net activity of the calpain system (µM calcium dependent calpain/calpastatin ratio), which by disassembling the myofibrils initiates degradation of myofibrillar proteins. µM-calpain also determines the rate of tenderization. We therefore hypothesize that the net calpain activity decreases and in turn affect the shear force of the meat by age. Thus, the aim of this study was to examine changes in shear force and gene expression (mRNA) of the calpain system (µM-calpain, mM-calpain, p94, and calpastatin) and the activity of µM-calpain and calpastatin in M. longissimus dorsi from the ages of d28 (8 kg) to d182 (145 kg).

II. MATERIALS AND METHODS

The experiment was carried out on 14 litters each of 3 castrated male pigs and 3 female pigs of the D(LY) breed. Within litter the pigs were slaughtered at 6 various ages ranging from d28, d70, d90, d140, and d182. Thus, the body weight

ranged from 8 to 143 kg live weight. The pigs were penned individually and had free access to a standard grower diet until d90 and then a finisher diet until day d182. Immediately after slaughter a muscle biopsy sample was taken from M. *longissimus dorsi* in the centre at the last rib curvature for measuring gene expression of calpains by RT-PCR. The day after a sample was taken from the last rib curvature and 20 cm caudally. These samples were condition for 2 days, frozen at 20 ^{o}C and stored until shear force analyses were carried out.

RNA extraction and RT-PCR

From muscle biopsy samples, RNA was extracted according to the trireagent extraction procedure. Purified RNA was reverse transcribed with oligo-dT primers and Superscript RNase H reverse transcriptase kit (Invitrogen, Taastrup, Denmark) and then amplified with TaqMan Univers al PCR Master Mix (Applied Biosystems, Stockholm, Sweden). Quantity of the mRNA from the µM-calpain , mM-calpain, p94, and calpastatin we used an ABI 7900HT detection system (Applied Biosystems, Stockholm, Sweden). The relative mRNA quantity was calculated: quantity = 2^{-Ct}.

Zymography

The activity of µ-calpain was measured by casein zymography using 12.5% Criterion gels (Gezymo, 18W, 1.0, Bio-Rad laboratories). The method was described in Therkildsen et al. (2004), only in the present study we used pre-casted gels with 18 wells. Each well was loaded with 10 µl muscle fluid extracted from 0.7 mg muscle tissue from *M. longissimus dorsi*, and each gel was loaded with samples from one litter, each sample loaded in triplicate. The volumes loaded on the gels were optimised to determinate the activity of µ-calpain, thus even though the activity of m-calpain was visible at the gels, the bands were too weak to quantify.

Calpastatin inhibitory activity

The principle of the assay was described by Thompson et al. (2000). We used the commercially available bodipy-labelled casein (ENZCHEK-protease assay kit, E-6638, Molecular Probes) and µ-calpain purified from porcine erythocytes from Calbiochem (#2078129). The assays were done in dublicate in white COSTAR-plates with 96 wells. The inhibitory activity of calpastatin was calculated by subtracting the activity of µ-calpain in wells with sample containing calpastatin from the activity of µ-calpain in wells without sample (positive). Each well was loaded with 20 µl µ-calpain (60 ng/µl), 30 µl $CaCl_2$ (200 mM), and either 50 µl diluted sample containing calpastatin (30 µl sample + 20 µl extraction buffer) or pure extraction buffer (positive). The reaction was started by adding 50 µl bodipy-casein (5 µg/ml in 100 mM Tris-base, pH 7.8) to each well, mixed on minishaker, covered with foil and incubated at 30°C for 30 min. The reaction was stopped by adding 30 µl 62.5 mM EDTA to all wells and mixed. The fluorescence was read at a Wallac Victor^2 flourometer at an exitation of 485 nm and an emission of 535 nm. The inhibitory activity of calpastatin was expressed as fluorescence per mg of muscle tissue.

Determination of shear force

Samples for shear force were thawed for 24 hours at 4 ^{o}C, heated to a core temperature of 70 ^{o}C, cut into 5 cm long 1x1 blocks along the fibre direction on which 8 replicate shear force determination were performed.

In this section you explain clearly how you carried out your study.

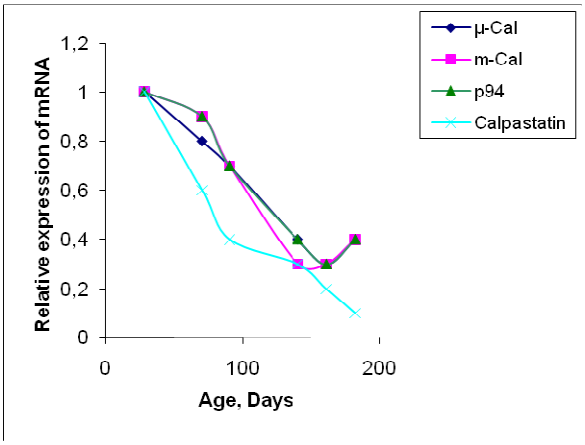
III. RESULTS AND DISCUSSION

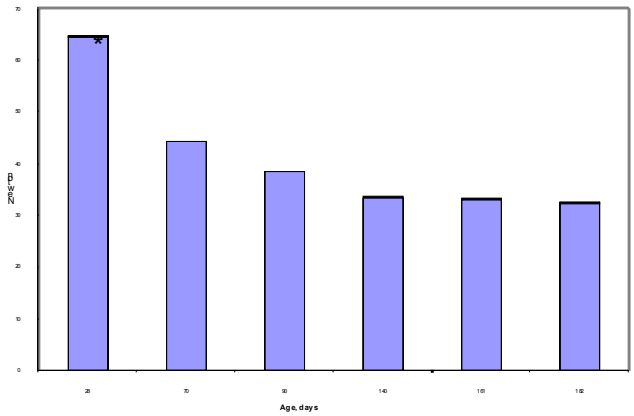
In Table 1, live weight and cold carcass are given at the various ages. The live weight varied between 8 kg at d28 and 146 kg at d182. Cold carcass weight varied between 6 kg at d28 and 114 at d182. The daily gain over the entire period was 895 g/day.

Table 1 Live weight and cold carcass weight of pigs slaughtered at 28, 70, 90, 140, 161 and 182 days of age

Age at slaughter, days	28	70	90	140	161	182	Root MSE	Effect of age	Effect of sex
Number of pigs	14	14	13	14	13	12			
No. castrated males									
No. females	7	7	7	7	6	4			
	7	7	6	7	7	8			
Live weight at slaughter									
Castrated males	8.2[a]	28.4[b]	45.1[c]	97.6[d]	120.7[e]	146.0[f]	8.9	***	NS
Females	7.7	29.3	46.9	99.0	122.8	145.7			
	8.6	27.5	43.2	96.1	118.7	146.2			
Cold carcass weight	5.8[a]	20.0[b]	31.5[c]	74.9[d]	94.0[e]	114.3[f]	8.7	***	NS

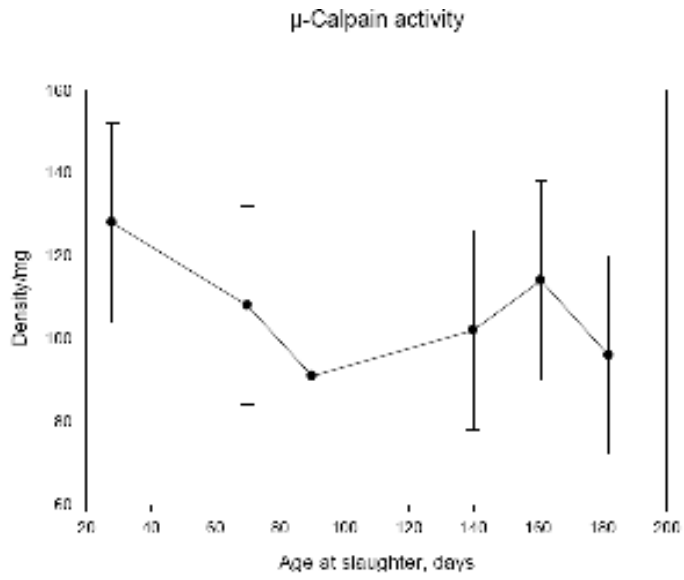
The results on Gene expression is shown in figure 1. From d28 to d140 gene expression of muscle calpains and calpastatin declined by increasing age. From d140 to d182 gene expression of calpains remained almost unchanged. However, the gene expression of calpastatin continued to decrease until d182 of age. Moreover, although not statistically verified, the relative decrease in calpastatin gene expression seems to be faster than for the calpains.

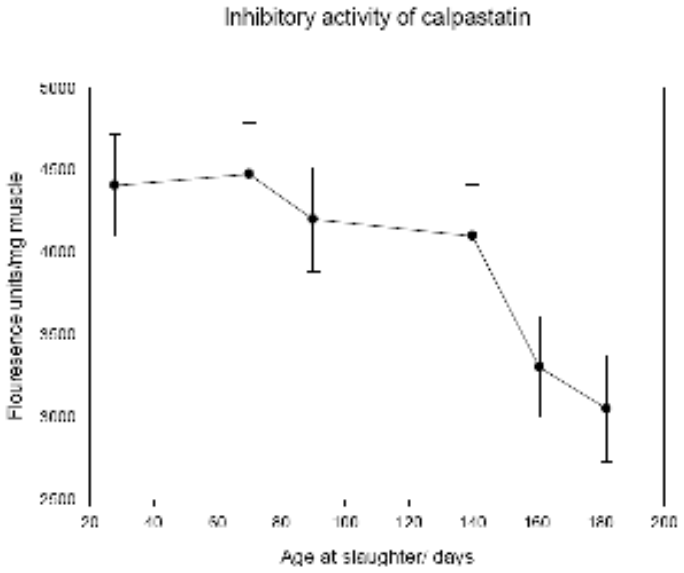




The results on the activity of μM-calpain and calpastatin are shown in figure 3 and 4, respectively. The activity of μM-calpain decreased from d28 to d90 and was almost unaltered until d182. In contrast, the activity of calpastatin was unaltered from d28 to d140 and decreased thereafter until d182. The initially relative decrease in mRNA of μM-calpain and a similar but faster relative decline in the mRNA of the inhibitor calpastatin and the early decline in the activity of μM-calpain and the unaltered activity of calpastatin support findings that the muscle protein turnover rate decline initially.

Shear force (Figure 2) of the meat declined by age from d28 to d90 and after this age no further changes by age was shown. This is in contrast to our hypothesis where we suggested the shear force to be lower at higher protein turnover. therefore However, other factors than the calpain contribute to shear force. Thus, in pig meat Intra-muscular fat exert a positive effect in the range from 1-3%. Although the collagen content may be unaltered by age, the solubility of the collagen may change and influence shear force. Sarcomere length was not measured in this experiment but Veiseth et al [3] found increased sarcomere length by age in lamb, which may have a positive effect on shear force by age. In older pigs, it seems that the proteolytic potential return to initial values. Thus form d140 to d182 mRNAs for μM-calpain are unchanged while the mRA for calpastatin continue to decrease. Similar results were obtained at the level of activity. This did not, however, cause changes in the shear force.





IV. Conclusion

We here report the result of an experiment where we examined the shear force and the calpain system at gene and activity level. Our data suggest that proteolysis initial (from d28 to d90) decline. In the same period also the shear force of longissimus declined. This is in contrast to our hypothesis suggesting that the rate of proteolysis is inversely related to shear force. Also at later ages the proteolytic activity returned to initial levels without changes in shear force.

References

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