DRIP LOSS INFLUENCES MUSCLE STRUCTURAL ANALYSIS OF THE PORCINE *LONGISSIMUS DORSI* POST MORTEM

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Introduction

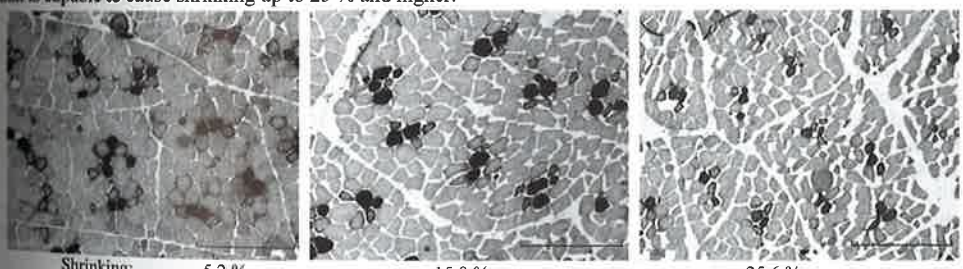
It is generally accepted, that proteolytic processes influence meat quality during time of ageing. These processes are also responsible for drip formation. In beef it was observed by Offer and Cousins (1992) that fibre bundles separate 4 to 6h *post mortem* from perimysium and within rigor mortis muscle fibres separate from epimysium while shrinking. That is caused by the degradation of the costameres, i.e. the cell membrane penetrating link between cytoskeleton and extracellular connective tissue (Taylor *et al.*, 1995). The emerging space between sarcolemma and cell body was identified as drip channels (Bertram *et al.*, 2004). M-calpain seems to be associated with β1-integrin and plays a significant role in their *post mortem* degradation (Lawson, 2004).
Considering this, the timing of sampling for histological analysis will effect morphometric analysis. The objective of this study was to quantify the shrinking of porcine *longissimus dorsi* muscle samples and how that affects the total muscle fibre count and cell density.

Materials and Methods

54 carcasses of commercially slaughtered pigs were investigated. They genetically derived from 3 genotypes. The sample includes both castrates and female pigs. Electrical conductivity (EC) was measured 24 h *post mortem* at 2nd/3rd last rib. Drip loss of 2.4 cm thick slices excised 30 h post mortem was measured after storage for 48 h at 4°C in polyethylene bags as described by Honikel (1987). *Longissimus* muscle cross sectional area was recorded. Muscle samples for histological investigations were taken 30 h *post mortem* and stored in liquid nitrogen. 12 µm thick cross sections were stained according to Horak (1983) to simultaneously identify fast twitch glycolytic (FTG), fast twitch oxidative (FTO) and slow twitch oxidative (STO) muscle fibres. Morphometric analysis was performed with the LUCIA software (Nikon). To yield the total amount of fibres per muscle as usual, single fibre diameters were recorded within a region of interest (ROI) followed by extrapolation to the *longissimus* cross sectional area. Shrinkage was determined measuring the area of primary muscle fibre bundles and subsequent subtraction of the summed area of the single measured fibres within that bundle. At 10 subsamples the actual fibre density was determined by counting all fibres within a ROI (9.87 mm²) also followed by extrapolation to the cutlet area to estimate the total fibre number of the muscle. Statistics were computed with SAS.

Results and Discussion

To illustrate shrinking, images of samples with varying drip loss are given in Figure 1. All samples show gaps between fibre bundles. The space between single fibres increases with rising drip loss. A high cellular damage must be assumed that is capable to cause shrinking up to 25 % and higher.



Shrinking:	5.2 %	15.0 %	25.6 %
Drip loss:	3.0 %	8.8 %	11.0 %

Figure 1: Porcine *longissimus* muscle cross sections stained for fibre typing (STO = dark; FTG = bright, FTO = bright with dark border) showing shrinkage related to the drip loss of the muscle sample (bar length = 500 µm).

Mean carcass traits and corresponding drip loss and shrinking can be seen in Table 1 demonstrating a great variation of the samples, e.g. drip loss or electric conductivity (EC). Shrinking extends from 1.1 % to 25.6 %.

Table 1: Basis statistics of carcass data, drip and shrinking (mean and standard deviation; n=54).

	Carcass weight, kg	Lean meat, %	*L. d.* area, cm²	EC, mS/cm	Drip loss, %	Shrinking, %
Mean	94.96	57,66	52.0	6,66	6,73	11,44
St.dev.	6.35	2,49	6.66	2,57	2.54	5,19

Correlations suggest FTG fibres to be more susceptible to shrinking compared with STO and FTO fibres (Table 2). With rising content of FTG fibres in muscle, exudativity would more likely occur. Muscle with higher frequencies of FTO and STO fibres would be more resistant in terms of generating purge. There is a tight relationship between drip loss and shrinkage of the cells (r = 0.66). Electrical conductivity is closely related both to the rate of shrinking and to drip loss and thus may be a good predictor.

Table 2: Correlations between histological parameters and carcass traits (n=54).

	Conductivity, mS/cm	Drip loss, %	Carcass weight, kg	Lean meat, %	Shrinkage, %
% FTG	0.17	0.41*	-0.03	-0.17	0,34*
% FTO	-0.09	-0,30*	-0.11	0,31*	-0,21
% STO	-0.17	-0,27*	0,22	-0.16	-0.29*
µm FTG	0,17	-0.30*	0,32*	0.29*	-0,31*
µm FGO	0,03	-0.10	0.05	0,16	-0.13
µm STG	0.29*	0,01	0,24	0,04	0.09
E. Conductivity		0,48*	0,31*	0.03	0,57*
Drip loss			-0.08	0.03	0,66*

* p<0,05

In Table 3 calculations of the total amount of muscle fibres and the cell density has been as usual and with consideration of the shrinking effect, respectively. If the counted mean cell density of 187 cells per mm² is declared as the gold standard, an overestimation occurs by simple calculating without considering the shrinking effect. The total amount of muscle fibres would be overestimated with 111,2 %.

Table 3: Estimated total amount of muscle fibres and cell density with and without considering shrinkage (mean and standard deviation; n=10).

	Shrinkage considered		Shrinkage not considered	
	counted cells / mm²	amount of muscle fibres	estimated cells / mm²	Total amount of muscle fibres
Mean	187	962,915	207	1,071,061
St.dev.	33,3	141389	38,3	182,932
Relation	100%	100%	110.9 %	111,2 %

Conclusions

Muscle fibre types susceptibility to shrinking is as follows: FTG>FTO>STO.
A high relationship between drip loss and shrinkage was observed.
Overestimation of the total amount of muscle fibres occurs if shrinkage was not considered.
A sampling within 45 min *post mortem* is recommended or biopsy has to be taken to most accurately measure fibre diameters and to calculate the most correct cell density.
Fibre bundles may be used to quantify shrinking and subsequently correct morphometric analyses, if autopsy sampling is inescapable.

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