Relationships between pH changes and extracellular space changes in *post mortem* muscle of calf.

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SUMMARY

The aim of this study was to investigate the relationships between pH changes (rate and extent) and extracellular space changes in *post mortem* muscles of calves.

Two experiments were designed. In experiment 1, large variations in the rate of pH fall were observed in the *Psoas major* muscle. At 4 h *post mortem*, pH varied between 5,60 and 6,40 ; these values corresponded to values of extracellular space (measured at 29 h *post mortem*) between 15% and 6%. pH fall and extracellular space were negatively correlated ($P < 0,01$). In experiment 2, variations in ultimate pH were induced by adrenaline administration. Ultimate pH values varied between 5,45 and 6,35 in the *Psoas major* muscle, and between 5,50 and 6,80 in both *Longissimus dorsi* and *Trapezius* muscles. Extracellular space, measured at 29 h *post mortem*, varied between 6,7 and 13% in the *Psoas major* muscle, between 4,5 and 10% in the *Longissimus dorsi* muscle and between 5 and 11% in the *Trapezius* muscle. Extracellular space was not correlated with ultimate pH, whereas it was with the rate of pH fall ($P < 0,01$).

It can be concluded that the extracellular space is influenced by the rate of pH fall, but probably not by the ultimate pH in veal muscle.

INTRODUCTION

Besides colour, which is generally the first criterion of evaluation for consumers and one of the most important factors determining the price of the veal carcass, tenderness and water holding capacity are two very important problems regarding veal quality.

Drip loss is positively correlated with the size of the extracellular space in *post mortem* pig muscle, at least at temperatures below 30 °C (PENNY, 1977). The increase in drip loss observed at higher temperatures would be due to protein denaturation (PENNY, 1977). PEARSON (1974) showed with nuclear magnetic resonance that water could be transferred from one region to another physically separated from the former. It has been suggested that *post mortem* changes in muscle could lead to a movement of water from the myofibrillar compartment into the sarcoplasmic compartment and into the extracellular space (PENNY, 1975 ; HONIKEL *et al*, 1986).

The aim of the present study was to investigate the evolution of the extracellular space during the onset of *rigor mortis* in muscle of veal calf. Relationships between the rate of pH fall and the extracellular space changes were studied in a first experiment ; in a second one, relationships between the ultimate pH and the extracellular space changes were evaluated.

MATERIAL and METHODS

Animals and sampling

Experiment 1 : 8 Friesan-Holstein calves originating from 2 farms were used. Four calves were artificially fed (skimmed milk powder and maize) and were kept in individual boxes, the other 4 animals suckled their mother.

The animals were bought when 18 week old (end of fattening) and transported to the Meat Research Laboratory in Theix (30 km in experiment 1, and 60 km in experiment 2). They were killed just after arriving by stunning and exsanguination. Fourty five minutes after slaughter, the muscle *Psoas major* was taken out. Two samples were cut from this muscle to measure pH and to estimate extracellular space. The rest was cut in pieces (50 to 100 g) and kept in a plastic bag at 25 °C in a waterbath. Every two hours (between slaughter and 10 h *post mortem*) and at 24 h *post mortem*, samples were taken from the pieces kept in the waterbath to measure pH and to estimate extracellular space.

Experiment 2 : 12 Friesan-Holstein calves, 18 week old, from a same fattening batch, were used. The animals were transported 2 by 2 to the Meat Research Laboratory in Theix (100 km far from the farm). One was killed just after arriving. The other one was injected with adrenalin around 3 h after arriving and again 6 h later, and killed 21 h after the first injection. Slaughter was made by stunning and exsanguination.

Thirty minutes and again 4 h and 29 h after slaughter, samples were taken from the *Psoas major*, *Longissimus dorsi* and *Triceps brachii*

muscles for pH measurement. One hour after slaughter, carcasses were put in a cold room at 4 °C. Twenty nine hours after slaughter, samples were taken from the three muscles to estimate extracellular space.

- *Analytical techniques*

pH measurement : 2 g of fresh meat were homogenized in 18 ml of 0,005M iodoacetate. pH was measured in the homogenate using a glass electrode.

Extracellular space measurement : bundles of fibres were frozen in pasty nitrogen at -210 °C. They were put in aceton for 3 days at -80 °C, then embedded in epoxy resin at ambient temperature. Cross-sections of 1,6 µm thickness were cut using an ultramicrotome (Ultracut E from Reichert). Then they were stained with toluidine blue (unspecific stain). The extracellular spaces were estimated from photographs of cross-sections (x = 300) by cutting and weighing.

- *Calculations* : linear regressions and variance analysis were used.

RESULTS and DISCUSSION

Experiment 1. Large variation in the rate of *post mortem* pH fall was observed but without relation with rearing conditions. Two animals, one from each farm, showed a very fast pH fall : at 4 h *post mortem*, pH values reached 5,59 and 5,68 (fig.1). However the ultimate pH values were similar in the eight carcasses of calves Extracellular space increased after slaughter. The ultimate value of extracellular space was almost reached at 10 h *post mortem* in all animals, and as soon as 6 h in the animal showing the fastest pH fall. From 4 h after slaughter, the two carcasses of calves with high rate of *post mortem* pH fall presented markedly higher extracellular spaces as compared with the others (fig.2). The values of extracellular space measured at 29 h *post mortem* were highly correlated with values of pH measured at 4 h *post mortem* (fig.3).

Experiment 2. A large variation in ultimate pH was obtained in the adrenalin-injected animals (table 1). It can be noticed that the injection affected the rate of pH fall : the pH of injected calves was higher than pH of control calves at 30 min and 4 h *post mortem*. The values of extracellular space are reported in table 2. The *Psoas major* muscle had a faster pH fall ($P < 0,01$) and a higher extracellular space ($P < 0,01$) than *Longissimus dorsi* and *Triceps brachii* muscles. Extracellular space as measured at 29 h *post mortem* was highly correlated with rate of pH fall as estimated by pH values at 30 min and 4 h *post mortem* (fig.4). On the other hand, extracellular space was not correlated with ultimate pH.

In experiment 1, the rapid pH fall occuring naturally in two calves reminds the case of PSE pork. To our knowledge, such a phenomenon has not been reported before in young calves. This fast pH fall was found in calves originating from two different farms, so it was not related to the conditions of rearing.

Extracellular space increases with time *post mortem* according to PENNY (1977). PENNY (1977) has found that the extracellular space tended to an upper limit of about 25% in *Longissimus dorsi* pig muscle. In the present study, extracellular space reached a maximum value of 15 % in the *Psoas major* muscle of one calf. These two values of extracellular space cannot be compared because of the differences in species (pig and calf), in muscle (*Longissimus dorsi* and *Psoas major*), and above all in techniques used to measure extracellular space. In the present study, samples were quickly frozen in a mixture of liquid-solid nitrogen, then water was freeze-substituted at - 80 °C. The sections were then embedded in epoxy resin before staining, so that it can be supposed that minor change occured in water distribution between freezing of the sample and measurement of the extracellular space. This was probably not the case in the study of PENNY (1977) since, in the technique used by this author, thawed unfixed sections were immersed in aqueous solutions for staining, so that changes in size of cells were possible during the staining process. Indeed, preliminary attempts to use classical histochemical techniques (no staining or haematoxylin and eosin or haematoxylin and trichrome, with or without fixation) showed us that the size of cells and extracellular spaces largely depend on the used technique.

A close positive relationship between the ultimate extracellular space and the rate of pH fall was previously reported by FIALIK (1983) in

pork. According to his observations, the extracellular space varied from 10 % in normal meat to 42 % in PSE meat in *Gluteus medius*, and from 11 to 26 % in *Gracilis* muscle. As discussed above, the large difference between these values and those found in the present study could be explained essentially by the difference in techniques.

CONCLUSION

Very rapid *post mortem* pH fall can occur sometimes in veal muscle. Extracellular space increases after death in muscle. The extracellular space, as estimated after completion of pH fall, is correlated to the rate of pH fall, but apparently not to the value of ultimate pH.

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Figure 1. Evolution of pH after slaughter in *Psoas major* muscle of calves

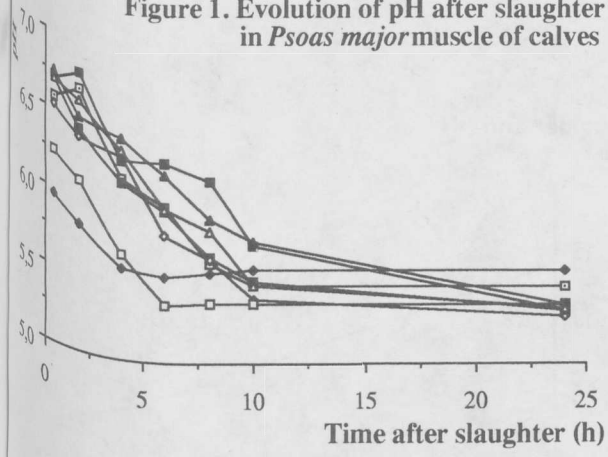
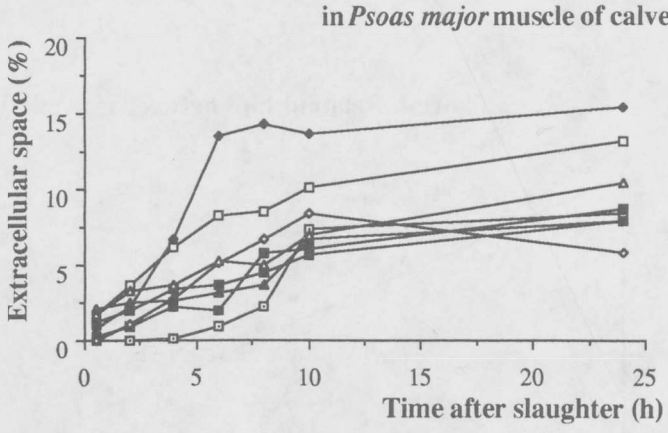


Figure 2. Evolution of the extracellular space in *Psoas major* muscle of calves



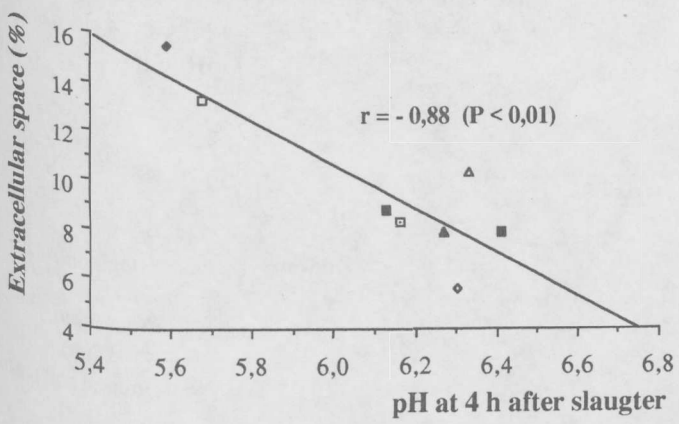


Figure 3. Relationship between pH measured at 4 h after slaughter and extracellular space measured at 29 h after slaughter in *Psoas major* muscle of calves

Table 1. Influence of adrenalin administration on *post mortem* pH changes

Time		*Longissimus dorsi*		*Trapezius*		*Psoas major*	
post mortem		Control	Adrenalin	Control	Adrenalin	Control	Adrenalin
30 min	mean	6,68	6,77	6,66	6,76	6,17	6,23
	s.e.	0,04	0,05	0,04	0,05	0,11	0,09
4 h	mean	6,53	6,53	6,40	6,51	5,75	6,30
	s.e.	0,05	0,11	0,04	0,04	0,17	0,10
29 h	mean	5,59	6,25	5,72	6,56	5,61	6,02
	s.e.	0,04	0,15	0,05	0,10	0,10	0,13
	min. / max.	5,48	6,72	5,60	6,80	5,46	6,35

Difference between adrenalin treated and control was significant at $P < 0,01$ for every muscle and every time except Ld at 4 h.

Table 2. Extracellular space values measured at 29 h after slaughter (%)

Extracellular space	*Longissimus dorsi*	*Trapezius*	*Psoas major*
mean	7,32 [a]	6,67 [a]	9,62 [b]
s.e.	1,55	1,68	1,65
min. / max.	4,53 / 10,02	4,73 / 10,76	6,73 / 12,99

Means with different superscript are significantly different.

Figure 4. Relationships between extracellular space and pH