ANAEROBIC GLYCOLYSIS RATE AND PSE CONDITION IN PORCINE LONGISSIMUS DORSI MUSCLE

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SUMMARY: Extreme PSE condition was observed in the muscles which had a large amount of lactic acid and a low pH within 2hr post mortem. At that stage they showed no fibres with glycogen and phosphorylase activity probably because all glycogen had been rapidly broken-down. A moderate PSE condition was found in the muscles which showed this pattern within 6hr pm. Therefore, the score of the PSE condition seems related to the rapidity of lactic acid accumulation and pH fall to low values, which in turn depend on the anaerobic glycolysis developing immediately after and/or before death. Moreover, the PSE condition is related to the presence of a large amount of glycogen in muscles before slaughter and to an effective phosphorylase activity, which provide substrate for anaerobic glycolysis. The difficulty in explaining the so-called late PSE condition has also been enphasized.

INTRODUCTION: The PSE pigmeat is related to an intensive anaerobic glycolytic rate in muscle which produces a large amount of lactic acid in a short period of time and therefore leads to a rapid pH fall after the death of the animal. The decreasing of the pH value after death in PSE muscles is faster than in normal ones and some researches have observed that the pH value at exsanguination is low in PSE muscles probably because an increased metabolic activity before death produced a certain amount of lactate (Löwe et al., 1977; Sjöblom and Lundström, 1989).
Our research has the purpose of investigating if and how the onset of PSE condition is affected by the length of time the muscle needs to reach the final low pH and how this process depends on the glycogen content, the efficiency of the glycogenolytic rate producing glucose-1-phosphate and the extent of the anaerobic glycolysis which leads to lactic acid accumulation.

MATERIALS AND METHODS: Longissimus dorsi muscles from 34 healthy cross bred pigs (Landrace x Large White) were used in this experiment. The animals weighing 120-140Kg were slaughtered at a commercial slaughter house after stunning by means of electronarcosis.
The L. dorsi muscle was isolated from the right side of the carcass within about 50 min after death, kept at room temperature (12°-16°C) until 2hr post mortem and then stored at 4°C. Samples taken at 1hr, 2hr, 6hr, 8hr, 12hr and 24hr pm were used for analyses.
The pH was measured by a pH-meter using 10g of muscle homogenised in 50ml of 5mM neutral iodacetate solution. The water holding capacity (WHC) measurement was carried out according to the filter-paper absorption method and was expressed as ratio value of meat film area to fluid area. Samples taken from the core of the muscle were frozen in liquid nitrogen and then equilibrated at -20°C and sectioned for histochemistry or stored

at -70°C until extraction with 1.0M perchloric acid and neutralization with 2.0M potassium carbonate for lactic acid determination by using the Automatic Clinical Analyser II (Du Pont Instruments U.S.A.). Serial sections of frozen muscle (16μ, myofibres transversely cut) were either fixed in Gendre solution and stained with periodic-acid Schiff (PAS) to evaluate the percentage of fibres with glycogen or placed in different incubating media to determine the percentage of fibres showing phosphorylase activity. To this purpose, the following substrate solutions, made according to Pierini et al. (1970) who modified the Takeuchi and Kuriaki (1955) method, were used: basic solution of 37.5mg glucose-1-phosphate and 5mg glycogen dissolved in 10ml of 0.2M acetate buffer, pH 5.6, (Medium 1) to which 2mg sodium fluoride (Medium 2) or 4mg AMP (Medium 3) or 8mg ATP and 4mg magnesium sulfate (Medium 4) were added. The above media were also prepared with 35% alcohol. Control sections were incubated in solutions without glucose-1-phosphate. After 20 minutes incubation at 37°C, the sections were immersed for 10 min in diluted Gram's iodine iodide solution and mounted on clean slides with iodine glycerin.
The colour was evaluated measuring the L* value with a Minolta Chroma Meter II reflectance colorimeter on a freshly cut surface. The wet appearance and the firmness were evaluated by a panel of three trained people. The following score was adopted: 1 = normal; 2 = moderately wet/soft; 3 = wet/soft; 4 = exudative/very soft.

RESULTS: The muscles were classified into six groups according to the most relevant characteristics observed at different times post mortem. Muscles belonging to groups 1 and 2 had very similar characteristics at 1hr pm (Table 1). The only difference was that those in group 2 had a larger number of fibres with a positive PAS reaction and phosphorylase activity. In all muscles the PAS-positive fibres surrounded the -negative fibres grouped in isolated islands. The phosphorylase activity was slightly increased by the presence of NaF but greatly by the presence of AMP or ATP and $MgSO_4$ in the medium. This means that the fibres contained both phosphorylase a and phosphorylase b. The majority of the fibres were blue-green in colour while few of them brown or green. The presence of alcohol increased colour intensity after incubation in all solution except Medium 4. The positive fibres were stained blue in the medium containing AMP and brown in the other media. A close relationship between the intensity of the phosphorylase activity and that of the PAS reaction was found in almost all myofibres. Probably as a consequence of the larger amount of total glycogen, the muscles in group 2 reached the final pH later (between 12 and 24 hours after death) than those in group 1 (between 8 and 12 hours pm) and showed a lower final pH and a higher lactic acid content. The number of fibres with PAS reaction and phosphorylase activity decreased, along with the intensity of these reactions, during the post mortem period until the final pH was reached. At that time very few and isolated or no fibres with glycogen and phosphorylase activity were detected. In many cases the phosphorylase a activity had ceased some hours before that of phosphorylase b. At 1hr pm the muscles in both groups had normal appearance (colour, wetness and softness) however at 24hr

TABLE 1

pH value, WHC value, lactic acid content (μmol/g of fresh tissue), percentage of fibres with PAS reaction and phosphorylase activity and appearance score (colour, wetness, softness) of muscles at initial and final stages post mortem.

GROUP		1	2	3	4	5	6
NUMBERS OF MUSCLES		6	3	2	8	6	9
characteristics at the initial stage	hours pm	1	1	1	1	1	1
	pH	>6.20	> 6.20	> 6.20	6.19-6.0	5.99-5.8	<5.70
	WHC	>2.0	> 2.0	> 2.0	> 1.5	> 1.5	<1.5
	lactic acid*	42-66	42-52	40-54	66-76	60-76	82-108
	% PAS+ fib.*	40-52	50-60	70	35-50	25-40	0-5
	% ph+ fib. *	40-52	50-60	70	35-50	25-40	0-5
	colour	1	1-2	2	2	2	3-4
	wetness	1	1	1	2	2	3-4
	softness	1	1	1	2	2	3-4
characteristics at the final stage	hours pm	12	24	24	6-8	6	1-2
	pH	< 5.80	< 5.60	< 5.60	< 5.70	< 5.70	< 5.70
	WHC	< 1.5	< 1.5	< 1.0	< 1.5	< 1.0	<1.0
	lactic acid*	80-90	96-102	98-110	94-108	92-106	96-110
	colour	1-2	2	4	2-3	3	4
	wetness	1	1	3	3	3-4	4
	softness	1	1	4	3	3	4

* extreme values.

pm those in group 2 were slightly pale.
A very peculiar condition is represented by the two muscles in group 3. Like the normal muscles they showed high pH and WHC and a small amount of lactic acid at 1hr pm, but were slightly wet, moderately pale and had a very large number of fibres with glycogen and phosphorylase activity. The intensity and distribution of the phosphorylase activity in the sections incubated in the media without alcohol were quite similar to those of muscles in groups 1 and 2, but brown-stained fibres were absent. Moreover, the presence of alcohol in the incubating solutions failed to augment the stainable reaction. The positive fibres were brown in colour. Fibres with glycogen and phosphorylase activity were found up to 12hr pm. The muscles showed the lowest pH at 24hr post mortem. At that time the values of the pH and WHC were very low, the lactic acid content was very high, no fibres with glycogen or phosphorylase activity were detected and muscles appeared extremely pale, soft and exudative (PSE) like muscles in group 6.
However, muscles in group 6 were extremely PSE as early as 1-2hr pm, when they showed the low final pH, a low WHC, a large amount of lactic acid and no fibres with glycogen and phosphorylase activity. In two cases, at 1hr pm a few fibres showed weak PAS reaction and phosphorylase activity only in the sections incubated in Media 3 and 4 without alcohol (blue colour) and in all media with alcohol (brown colour).
On the other hand, muscles in groups 4 and 5 reached a final metabolic condition similar to that in group 6 (low pH and WHC, high lactic acid content, no fibres with glycogen and phosphorylase activity) in a longer period of time (within 6-8hr pm) and at that stage they appeared only moderately PSE. At 1hr pm these muscles had a pH between 5.80-6.16, were slightly pale, wet and soft and showed a lower number of fibres with glycogen and phosphorylase activity than muscles in groups 1 and 2 (normal muscles).

DISCUSSION AND CONCLUSIONS: Swatland and Cassens (1973) found that L. dorsi muscle of extremely stress susceptible pigs had an increased number of myofibres with a positive PAS reaction at the time of exsanguination. Lindholm et al. (1979) observed a higher proportion of fast twitch low oxidative fibres and a higher glycogen content in L. dorsi muscle of stress susceptible pigs.
Our results show that PSE condition was observed only in the muscles which had a large amount of lactic acid at the time they reached the final pH. This means that they had a high content of glycogen immediately before slaughter and a very efficient glycogenolysis and anaerobic glycolysis. Therefore, an effective breakdown of a large amount of glycogen is essential to cause PSE characteristics in meat. However, we found an extreme PSE condition in the muscles showing no fibres with glycogen and phosphorylase activity at 1-2 hours post mortem and a moderate PSE condition in those showing no fibres with glycogen and phosphorylase activiy within 6hr pm, corresponding to, in both cases, the times they presented the lowest pH and WHC and the highest content of lactic acid. The relatively low pH and high content of lactic acid at 1hr pm indicate

a very rapid glycogenolysis after exsanguination.
Considering these data and the observations made on normal muscles, we assume that these muscles had an accelerated glycolgenolytic and glycolytic activity within a few hours post mortem. Swatland and Cassens (1973) detected fibres with an intense PAS reaction and phosphorylase activity in muscle of stress susceptible pigs at 20 minutes after death, but did not observe such fibres in samples taken 45 min pm from pigs which died as a result of their extreme SS condition and the stress applied to them. In our case, the phosphorylase activity in PSE muscles was not detected at 2hr pm probably because all glycogen had been rapidly broken-down after which the metabolites inhibited the enzyme activity. Schwägele and Honikel (1988) observed that total and specific activities of phosphorylase a + b in muscles with pH1-values between 5.5 and 6.1 was five times higher than in those with pH1 above 6.1.
The rapid glycogenolysis rate post mortem leads to a fast drop in pH when the temperature in muscle is still high and causes denaturation of protein, degeneration of membranes and therefore PSE meat (Bendall and Wismer-Pedersen, 1962; Briskey, 1964; Honikel and Kim, 1985). Nevertheless, a lower pH at the time of exsanguination might contributed in initiating the development of the faster glycogenolysis rate (Sjöblom and Lundström, 1989).
The above considerations regarding the onset of the PSE condition, however, do not explain the behaviour of the muscles in our group 3. These showed a slow decrease in the pH and in the number of fibres with a PAS and phosphorylase reaction, but were very pale, soft and wet at 24hr pm. Eventhough their behaviour within the first few hours pm was very different from that of the other PSE muscles, they had final PSE characteristics. This was well expressed also by the lesser weight loss during the 24-hour period after death. The question arises whether they must be considered truly PSE and why they were so pale and wet.

REFERENCES:

Bendall, J.R. and Wismer-Pedersen, J. (1962). J. Food Sci. 27:144.
Briskey, E.J. (1964). Adv. Food Res. 13:89.
Honikel, K.O. and Kim, C.-J. (1985). Fleischwirtsch. 65:125.
Lindholm, A., Persson, S., Jonsson, L. and Andrén, H. (1979). Acta Agric. Scand., 21:357.
Löwe, G., Stainhardt, M., Beutling, D., Lyhs, L. and Fachmin, G. (1977). Arch. Exper. Vet. med., Leipzig, 31:643.
Pierini, B., Splendiani, F. e Rampichini, L. (1970). Ann. It. Derm. Clin. Sper. 24:3.
Schwägele, F. and Honikel, K.O. (1988). 34th Internat. Congress Meat Sci. & Technol. B:575.
Syöblom, A.-C. and Lundström, K. (1989). 35th Internat. Congress Meat. Sci. & Technol. 3:161.
Swatland, H.J. and Cassens, R.G. (1973). J. Animal Sci. 37;885.
Takeuchi, T. and Kuriaki, H. (1955). J. Histochem. and Cytochem. 3:153.