3:33 The relationship between water holding capacity, pH and glycolysis rate in the swine muscle post mortem

M. SEVERINI, A. VIZZANI and G. CENCI

Istituto di Ispezione degli Alimenti di Origine Animale, Facoltà di Medicina Veterinaria, Università degli Studi, Perugia, Italia

Introduction

The PSE muscles of pigs show a rapid fall in pH after slaughter while the carcass is still warm and this condition seems to reduce the water holding capacity (WHC). We have observed that this WHC is very important in the aging process of dry-cured ham. In fact a very low WHC 24 hours after death favours the occurrence of defects during aging (Di Antonio, Severini, Vizzani e Cenci, 1982; Severini, Di Antonio, Vizzani, Cenci e Avellini, 1983). Furthermore we noted the existence of a relationship between WHC 1 hour and pH 1 hour after death and also between WHC-1 hr and the pH value found after 1 hr and after 24 hr. However we also found a certain number of cases in which though the pH-1 hr was low, the WHC-1 hr was greater than that predicted and the PSE condition was not evident (Severini, Vizzani, Cenci e Bertorotta, 1983). The fall in pH and the drip loss post mortem seem to be the result of a rapid glycolysis within the first hour after slaughter (Bendall and Wismer-Pedersen, 1962; Sybesma and Eikelenboom, 1969). A low pH and high temperature cause denaturation of muscle proteins and formation of a large amount of drip (Bendall and Wismer-Pedersen, 1962). Fischer, Hamm and Honikel (1979) have studied the changes in solubility and enzymic activity of glycogen phosphorylase in order to evaluate the denaturation of proteins in PSE muscle. The phosphorylase activity has also been studied histochemically in order to identify myofiber populations in PSE muscle (Swatland and Cassens, 1973).

The aim of the present work is to investigate the relationship between the PSE condition, the pH value and the WHC 1 hr after slaughter; to evaluate the prevalence of carcasses with PSE or abnormal muscle of pigs slaughtered for dressed pork products; to examine the relationship between pH-1 hr, WHC-1 hr and the rate of glycolysis by histochemical (glycogen amount and phosphorylase activity) and biochemical (lactic acid amount) tests.

Materials and Methods

Two hundred and four (204) carcasses of healthy pigs (male and female, mixed breed L x LW, live weight 140-160 kg) slaughtered and dressed at the same slaughterhouse were used in this experiment.
About 60 min after slaughter the pH (pH-1), temperature and water holding capacity (WHC-1) were carried out on the freshly cut cross-section of Longissimus dorsi muscle between the 5th-6th ribs of the right side of the carcass.

The pH-1 was measured at three different locations with a pointed glass electrode using a pH meter Top Tronic. The temperature was determined using a Top Tronic electric thermometer. Water holding capacity (WHC) measurement was carried out according to the filter-paper absorption method of Grau-Hamm (1957) on a muscle sample of about 0.300 g. The WHC was expressed as ratio value of meat film area to fluid area (measured with a planimeter).
A sample weighing about 200 g was also taken from twenty five Longissimus dorsi muscles 1 hr after death. A subsample was immediately frozen in liquid nitrogen and stored at -70°C until sectioned for histochemistry; a second subsample was frozen and stored at -70°C until extraction with 1.0M perchloric acid and neutralisation with 2M KOH, pH 10-11; the remainder was placed in a polyethylene box and stored at about 10°C. Subsamples were taken from this fresh portion of muscle 120 min after death and frozen as previously described for histochemical and biochemical tests or used for WHC-2 (2 hours after death) and pH-2 measurement. The pH-2 (2 hours after death) was determined with a radiometer pH meter using 10 g of muscle homogenised in 50 ml of 5mM neutral iodoacetate solution.
To determinate the phosphorylase activity serial sections (16µ, myofibers transversely cut) were incubated for 20 min at 37°C in different media buffered to pH 5.8 and containing glucose 1 phosphate and glycogen only (medium 1) or with NaF (medium 2), AMP (medium 3), ATP and $MgSO_4$ (medium 4). The control media were without glucose 1 phosphate. The reaction product was stained in a weak iodine solution. To determine intrinsic glycogen the serial sections (10µ) were fixed for 5 min in a Gendre solution at 0°C and were stained with the PAS reaction according to the McManus method (with α and β amylase control). All the histochemical tests were carried out according to the methods of Pierini, Splendiani, Rampichini (1970). The lactic acid was determined in the extract using the Automatic Clinical Analyser II (Du Pont Instruments U.S.A.). We observed the colour appearance in 145 muscles.

Results

Table 1 shows the distribution of the cases in different groups of pH-1 and the average values of the WHC-1 and of the temperature for each group. We can see that almost 60% of the muscles had a pH-1 ≤ 6.0 and that as the pH-1 value decreases the average value of WHC-1 also decreases. The average values of the temperature are high for all the pH-1 groups and the temperatures are slightly higher in the groups with the pH-1 ≤ 5.8.
Table 2 shows the distribution of the cases according to the pH and WHC observed 1 hour after death. The muscles with pH-1 ≥ 6.2 all have WHC-1 > 1.50 and 97.6% have a WHC-1 > 2.00. The number of muscles with WHC-1 ≤ ≤ 2.00 or with WHC-1 ≤ 1.50 gradually increases as the pH-1 value of each group decreases. 69.0% of the muscles with pH-1 ≤ 5.59 have a WHC-1 ≤ 1.00.
Table 3 shows the distribution of cases according to the pH and colour appearance observed 1 hour after death. The percentage of muscles with a more or less pale appearance increases as the pH-1 value of each group decreases.

Muscles with an extremely pale appearence all had a pH-1 ≤ 5.59.
Table 4 shows the distribution of cases according to WHC and colour appearance observed 1 hour after death. The percentage of muscles with a more or less pale appearance increases as the WHC-1 decreases. 87.5% of muscles with an extremely pale appearance have a WHC-1 ≤ 1.00 and 12.5% have a WHC-1 ≤ 1.50. The 11.0 percent (16/145) of the muscles examined for colour appearance have pH-1 ≤ 5.59, WHC-1 ≤ 1.50, an extremely pale appearance and they already have a clear PSE condition 1 hour after death.

TABLE 1

DISTRIBUTION OF CASES ACCORDING TO pH 1 HOUR AFTER DEATH

pH	n°	%	WHC-1	T°
≥ 6.20	41	20.1	6.19 ± 4.41*	40.7 ± 0.8
6.0-6.19	41	20.1	4.10 ± 4.05*	40.9 ± 0.7
5.8-5.99	50	24.5	2.23 ± 2.00*	40.9 ± 0.9
5.6-5.79	43	21.1	2.01 ± 0.90	41.3 ± 0.6
≤ 5.59	29	14.2	0.94 ± 0.40	41.5 ± 0.9
TOTAL	204			

* The high standard deviation value is due to the presence of very high WHC values.

TABLE 2

DISTRIBUTION OF CASES ACCORDING TO pH AND WHC 1 HOUR AFTER DEATH

pH	WATER HOLDING CAPACITY ≤ 1.00	1.01-1.50	1.51-2.00	≥ 2.01	TOTAL
≥ 6.20	0** 0* 0***	0 0 0	1 2.4 2.4	40 97.6 40.0	41
6.0-6.19	1 2.4 4.0	5 12.2 13.2	7 17.1 17.1	28 68.3 28.0	41
5.8-5.99	2 4.0 8.0	16 32.0 41.1	13 26.0 31.7	19 38.0 19.0	50
5.6-5.79	2 4.6 8.0	12 27.9 31.6	16 37.2 39.0	13 30.2 13.0	43
≤ 5.59	20 69.0 80.0	5 17.2 13.2	4 13.8 9.4	0 0 0	29
TOTAL	25 12.3	38 18.6	41 20.1	100 49.0	204

* Number of cases; ** percentage of the total in the column; *** percentage of the total in the line.

Table 5 shows the distribution of 25 cases (histochemically and biochemically tested) according to pH and WHC observed 1 hour after death. The muscles with pH-1 ≥ 6.2 had (1 hour after death) a normal colour, a WHC-1 greater than or close to 2.00 and an average value of 59µmol of lactic acid/g of fresh tissue. All the muscles had 50-60% of myofibers with quite a large quantity of stainable glycogen. Phosphorylase activity was detected in 50-55% of myofibers mostly located at the periphery of fasciculi. Most of these myofibers had a blue/greyish-green colour and a few had a slightly brown colour. The presence in the medium of NaF increased the intensity of the colour slightly. When the sections were incubated with AMP in the medium the colour intensity and the number of positive myofibers, increased greatly

TABLE 3

DISTRIBUTION OF CASES ACCORDING TO pH AND COLOUR APPEARANCE 1 hr AFTER DEATH

pH	COLOUR APPEARANCE normal	moderately pale	pale	extremely pale (PSE)	TO
≥6.20	16* 72.8 25.4	3 13.6 10.0	3 13.6 8.3	0 0*** **0	2
6.0-6.19	17 58.6 27.0	4 13.8 13.3	8 27.6 22.2	0 0 0	2
5.8-5.99	14 46.7 22.2	7 23.3 23.4	9 30.0 25.0	0 0 0	3
5.6-5.79	15 45.5 23.8	12 32.4 40.0	10 27.1 27.8	0 0 0	3
≤5.59	1 3.7 1.6	4 14.8 13.3	6 22.2 16.7	16 59.2 100.0	2
TOTAL	63 43.5	30 20.7	36 24.8	16 11.0	14

TABLE 4

DISTRIBUTION OF CASES ACCORDING TO WHC AND COLOUR APPEARANCE 1 hr AFTER DEA

WHC	COLOUR APPEARANCE normal	moderately pale	pale	extremely pale (PSE)	TO
≤ 1.00	0* 0*** **0	2 9.1 6.7	5 27.3 16.7	14 63.6 87.5	2
1.01-1.50	7 24.1 11.1	8 27.6 26.7	12 41.4 33.3	2 6.9 12.5	2
1.51-2.00	11 36.7 17.5	10 33.3 33.3	9 30.0 25.5	0 0 0	3
≥ 2.01	45 70.3 71.4	10 15.6 33.3	9 14.1 25.5	0 0 0	6
TOTAL	63 43.5	30 20.7	35 24.8	16 11.0	14

* Number of cases; ** percentage of the total in column; *** percentage of total in the line.

The reaction product appeared predominantly blue in colour. When sections were incubated with ATP and $MgSO_4$ in the medium, the brown colour of the few positive myofibers became more intense. Two hours after slaughter these muscles had a slightly lower pH-2, but still greater than 6.0, a WHC-2 still higher or close to 2.00 and a slightly greater average amount of lactic acid (85μmol/g). The number of the myofibers containing glycogen decreased on the average by 50-60% and the intensity of PAS reaction was also lower. The number of myofibers with phosphorylase activity decreased on the whole by 60-70% and the colour intensity also decreased in each section incubated in the various media.

The muscles with pH-1 ≤ 5.59 had 1 hour after slaughter WHC-1 ≤ 1.00, temperature greater than 41.5, a large amount of lactic acid (93μmol/g) and a pale or extremely pale appearance. All the muscles had only a few isolated myofibers with little PAS positive reaction. The phosphorylase activity was present in these myofibers only in the sections incubated with AMP in the medium; in a muscle this activity was slightly intense and diffuse. The reaction product was always of a pale greyishgreen colour. Two hours after death all these muscles had a pH-2 slightly higher, but still lower or close to 5.6, a WHC 2 hr ≤ 1.00 and a large amount of lactic acid (100μmol/g) similar to the amount observed at 1 hour. The glycogen and the phosphorylase activity were almost absent.

TABLE 5

DISTRIBUTION OF 25 CASES ACCORDING TO pH-1 AND WHC-1

pH	WATER HOLDING CAPACITY				TOTAL
	≤ 1.00	1.01-1.50	1.51-2.00	≥ 2.01	
≥ 6.20	-	-	-	5	5
6.0-6.19	-	-	1	4	5
5.8-5.99	-	1	2	1	4
5.6-5.79	-	2	-	4	6
≤ 5.59	5	-	-	-	5

The muscles with pH-1 6.0-6.19 had a normal or moderately pale colour. Four of them had a WHC-1 > 2.00 and an average value of 78μmol of lactic acid/g of fresh tissue (with a large range of values) 1 hour after death. The percentage of myofibers with glycogen and phosphorylase activity and the colour intensity of the reaction products were as high as in the muscles with pH-1 ≥ 6.2 though there was a wide variability between the muscles. After two hours these muscles had about the same pH, a WHC-2 > 2.00 or slightly greater than 1.0 and an average value of lactic acid equal to 73μmol/g (with a large range of values). The amount of glycogen and the phosphorylase activity decreased on the average by about 50-60%.

A muscle of this group had a WHC-1 and a WHC-2 ranging from 1.50 to 2.00 and a very low level of lactic acid 1 hour (36μmol/g) and two hours (42μmol/g) after death. The glycogen content and the phosphorylase activity were also very low 1 hour and 2 hours after slaughter. We believe that this muscle had a small quantity of glycogen ante mortem (probably due to stress) and quite a fast glycolysis.

The muscles with pH-1 5.8-5.99 showed a wide range of the various values. Two of these had a small amount of lactic acid 1 hour and 2 hours after death, WHC-1 close to 1.50 and WHC-2 ≤ 1.00. The content of glycogen and the phosphorylase activity after 1 and 2 hours were quite similar to the muscles with pH-1 ≤ 5.59. Another muscle with WHC-1 > 2.00 and WHC-2 > 2.00 was histochemically and biochemically quite similar to the muscles with pH-1 ≥ 6.2. A fourth muscle with WHC-1 and WHC-2 1.00-1.50 showed a very strong fall in pH 2 hours after death (pH 5.50) as well as a great increase in lactic acid (from 64μmol/g at 1 hour to 110umol/g 2 hours after slaughter). The amount of glycogen was very large 1 hour after death and the phosphorylase activity was quite diffuse in all the myofibers and was very intense in colour. After two hours the glycogen content decreased by 50-60% and the phosphorylase activity decreased by 30%. In this case it seems that glycolysis started later than the normal, but subsequently was very rapid.

The muscle with pH-1 5.6-5.79 showed different characteristics. Those with WHC-1 < 1.50 (very close to 1.00) were on the whole very similar to the muscles with pH-1 ≤ 5.59 though the amounts of lactic acid were slighly lower. The muscles with WHC-1 > 2.00 had a high WHC-2 and an average high level of lactic acid 1 hour (88μmol/g) and 2 hours (94μmol/g) after death. They were histochemically similar to the muscles with pH-1 ≥ 6.2, but the glycogen content and the phosphorylase activity were rather lower.

Conclusions

A high percentage of pigs examined showed rather low pH values 1 hr after death. 59.8% of the muscles had a pH-1 < 6.0 and 35.3% had pH-1 < 5.8. This high percentage could be related to seasonal factors as demonstrated by research carried out in Italy (Russo, Bosi e Casini, 1983). We found a clear PSE condition 1 hour after death only in those muscles with pH-1 ≤ 5.59 and WHC-1 ≤ 1.50 (11.0% of the 145 cases examined for colour as well). We also noted that 86.2% of the muscles with pH-1 ≤ 5.59 had a WHC-1 ≤ 1.50 and that 30.0% of all the muscles with pH-1 < 6.2 had a WHC-1 ≤ 1.50. Histochemical tests indicated that the glycogen and the phosphorylase activity almost completely disappeared 1 hour after death in muscles with pH-1 ≤ 5.8 and WHC-1 ≤ 1.50. At 1 hour post mortem the glycolysis in these muscles was early completed as shown by the fact that the levels of lactic acid were already high 1 hr post mortem and did not increase 2 hours after death (Monin, Ollivier, Goutefongea and Girard, 1981). However a few muscles with pH-1 5.8-5.99 and with pale or moderately pale appearance 1 hr after slaughter showed an early complete glycolysis 2 hours after death. In this case relatively low WHC-2 (2.00) was also reached. On the other hand muscles with pH-1 > 5.6 and WHC-1 > 2.00 had a slower disappearance of the glycogen and phosphorylase activity within the first 2 hours after death. The rate of this disappearance seems to be related to the pH-1 and the glycogenolysis seems slower in muscles with higher pH-1 values. This relationship is quite good but not always easy to evaluate because of the different distribution of the various types of myofibers on the cross-section of the muscle and because of the histochemical measurement of phosphorylase activity which depends also on the interpretation given to the colour of the reaction product (Swatland, 1978). The pH-1 therefore seems to be related to the glycogen content in the muscle just before slaughter as well as the rate of glycolysis post mortem depending on genetic (stress susceptibility of pigs) and environmental factors that cause stress (Monin, Ollivier, Goutefongea and Girard, 1981). The presence of a large amount of glycogen before the slaughter and a fast glycolysis post mortem are demonstrated by a rapid fall in pH, an early low WHC, a rapid increase of lactic acid level and an early decrease of glycogen content and phosphorylase activity. Fischer, Hamm and Honikel (1979) showed a diminuition of the solubility and activity of glycogen phosphorylase in PSE muscles. Cheah, Cheah, Crosland, Casey and Webb (1984) suggest that the enhanced glycolysis is associated with the elevated sarcoplasmic calcium levels in PSE meat.

When the rate of glycolysis is high and the amount of glycogen ante mortem is small the decrease of glycogen content and phosphorylase activity is rapid, but the muscle does not reach a high acidity as in our atypical case. It sometimes happens that the glycogen content is very large and that the glycolysis post mortem starts later than normal as in our second atypical case which resembles those observed by Swatland and Cassens (1973). On the basis of our findings we believe that the Longissimus dorsi muscles of pigs with pH-1 ≤ 5.8 and WHC-1 ≤ 1.50 can be regarded as PSE. The muscles with pH-1 ≤ 6.0 and WHC-1 ≤ 2.00 can be considered as moderately PSE. When the identification of Longissimus dorsi PSE muscles is based only on pH-1 value we believe that muscles with pH-1 ≤ 5.6 must be regarded as PSE given the high probability that they have a WHC-1 ≤ 1.50 which seems to be a critical level for the quality of these muscles 1 hour after death. This probability is greatly reduced for muscles with a higher pH-1, though it is still quite high for muscles with pH-1 ≤ 6.0. This probability is almost nil for muscles with pH-1 ≥ 6.2.

In conclusion, we believe that the PSE condition is difficult to define because it can be evaluated according to different physical, chemical, biochemical or histochemical tests, e.g. fall in pH, water holding capacity, rate of glycolysis, calcium release, colour appearance, etc.. The results of experimental and statistical studies can vary according to how we consider PSE condition and how we relate it to the stress susceptibility of pigs. This could explain the existence of the frequent occurrence of discrepancies. On the basis of these considerations we believe that the PSE condition in the muscles we examined is characterised by the rapid fall in pH, the rapid breakdown of glycogen by the phosphorylase activity, the rapid production of a large quantity of lactic acid, high temperature and low WHC just 1 hour after death. The critical pH and WHC levels probably vary according to the breed, sex and the age of the pigs, the breeding, transport and slaughter conditions and the season. We found the PSE condition in a high percentage of muscles with pH-1 ≤ 5.59 and WHC-1 ≤ 1.50. However, the characteristics of glycolytic metabolism of these muscles were also present in muscles with pH < 5.8 and WHC ≤ 1.50 which had a pale appearance. 19.12% of the total muscles examined therefore had a glycolytic rate, fall in pH and WHC similar, if not identical to these muscles with a clear PSE condition and can be regarded as PSE muscles.

Summary

Two hundred and four Longissimus dorsi muscles of pigs were examined. 19.2% showed pH < 5.8, WHC ≤ 1.50, rapid glycolysis post mortem and pale or extremely pale colour appearance 1 hour after death (PSE muscles). A high percentage of muscles showed pH-1 hr 5.8-6.19 and quite a variation in WHC-1 hr and rate of glycolytic metabolism.

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