INFLUENCE OF LOW-VOLTAGE STIMULATION ON POST MORTEM BIOCHEMISTRY IN NORMAL AND DFD BEEF

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INTRODUCTION

The Swedish method of low voltage stimulation (LVS) of beef carcasses (MITAB), is now established in most Swedish abbatoirs and in several European countries. Like high voltage stimulation (HVS) LVS has been shown to increase the rate of glycolysis after slaughter in normal carcasses, measured as faster pH decline (Fabiansson et al. 1979, Rudérus 1980). resulting in the same ultimate pH as in unstimulated carcasses it has also been shown to lessen the development of tenderness (Buchter 1980, Rudérus and Fabiansson 1980). HVS has also been shown to give rapid ATP depletion, as well as rapid pH decline (Bendall 1980), and to give a brighter red colour and a better flavour than unstimulated carcasses (Smith et al. 1980). Most of the research so far on electrical stimulation has been focused on trying to explain the mechanisms of the tenderizing effect, as reviewed by Cross (1979), Bendall (1980) and Dutson (1980).

The influence of electrical stimulation on dark cutting beef (DFD) was studied by Sorinmage et al. 1978, who found that stimulation does little to enhance tenderness of stressed animals, and Dutson et al. (1980) who found no improvement in muscle colour of DFD beef. DFD is a common problem in most countries causing problems as dark colour, firm and dry consistency, faster spoilage (Tarrant 1980), unacceptable flavour development (Dutson (1980) and less nonenzymatic browning reactions during frying (Laser Reuterswärd, unpublished results). No documentation is available concerning the influence of LVS on DFD. Thus an investigation was carried out in order to study the effects of LVS on the incidence of DFD and on different meat quality aspects of DFD compared to normal beef (Jelkner Modig and Rudérus 1981, Erichsen and et al. 1981). The purpose of this part of the investigation was to study the contents of some metabolites in glycolysis and from ATP degradation, which are especially important for pH decline, nonenzymatic browning and flavour development.

MATERIAL AND METHODS

The investigation was carried out on 32 young bulls at a slaughter weight ranging between 240 and 400 kg. 16 of the bulls were exhausted before slaughter according to Jelkner-Modig and Rudérus (1981) and the other 16, which were gently treated, were used as a control group. 8 bulls in each group were electrically stimulated (85V, 32s, 14Hz) immediately after exsanguination (Rudérus 1980). About one h after slaughter the carcasses were chilled at +2°C for 2 h and then kept at about +5°C for two days.

The pH was measured and samples were taken from the M. longissimus dorsi opposite the second lumbar vertebra 1 h and 42 h after slaughter respectively. pH was measured with a portable pH-meter (Knick Portamess 651) with a glass electrode (Ingold 404T) at a depth of 2 cm. A few cm from the place where pH was measured samples of about 6 grams were taken with a stainless cylinder (diameter 2 cm, lenght 5 cm). The outer part (1 cm) of the meat sample was cut off and the remainder was immediately frozen in liquid nitrogen. Within 2 h the frozen samples were homogenized with percloric acid and centrifuged at 10 000 x g for 20 minutes. The supernatant was adjusted to pH 4.0 with KOH and the extract was used for analysis of lactate, glycogen, glucose, glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), ATP, ADP, AMP, IMP, inosine and hypoxanthine. All metabolites are expressed in µmol/g tissue. Lactate was determined with test-combination Cat. No 139 084 from Boehringer Mannheim. Lactate was determined by the enzymic method of Keppler and Decker (1974). Glucose, G6P and F6P were determined by the enzymic methods of Bergmeyer et al. (1974) and Bernt and Bergmeyer (1974). Nucleotides, inosine and hypoxanthine were separated on a Waters 6000 HPLC equipment and detected by UV absorption at 254 nm. ATP and ADP were separated on a µ Bondapak-NH_2 column (Waters Associated) eluted with 0.125M $(NH_4)H_2PO_4$ + 0.004M KCl, pH 5.0 at a flow rate of 1.6 ml/min. AMP and IMP were separated on a partisil SAX anion exchanger (Whatman) eluted with 0.03M $(NH_4)H_2PO_4$, pH 3.4 at a flow rate of 1.1 ml/min. Inosine and hypoxanthine were separated on a µ Bondapak-C18 column (Waters Associated) eluted with 0.005M $(NH_4)H_2PO_4$ + 0.005 M KCl, pH 3.0 at a flow rate of 1.5 ml/min. The data were analysed by Student's t-test on a Hewlett Packard 9825 desk top computer

RESULTS AND DISCUSSION

Depending on ultimate pH the muscle samples were assigned to different classes (Fischer and Hamm 1980), samples with pH ≤5.80, 5.81 ≤ pH ≤6.20, and pH ≥6.21 being designated as normal, medium, and DFD respectively. All samples from gently treated animals, both stimulated and unstimulated, were classified as normal. Of the exhausted animals 4 stimulated and 4 unstimulated were classified as DFD and thus the incidence of DFD was not affected by stimulation. One exhausted stimulated animal with an ultimate pH <5.80 was classified as medium (for reasons given below). The remaining 7 animals were classified as medium. Thus we ended up with 6 classes or groups of samples designated unstimulated normal, stimulated normal, unstimulated DFD, stimulated DFD, unstimulated medium and stimulated medium. The results are reported in table 1 and 2 as means with standard deviations for the different groups. However, for reasons given below the stimulated medium group is not included in the tables.

Table 1. Contents of metabolites 1 h post mortem (µmol/g tissue), means with standard deviations.

	Normal Group		DFD Group		Middle Group
	Unstimulated n=8	Stimulated n=8	Unstimulated n=4	Stimulated n=4	Unstimulated n=4
pH	6.58 (0.34)	5.96 (0.04)	6.75 (0.19)	6.84 (0.03)	6.62 (0.23)
Lactate	12.42 (2.44)	46.84 (8.64)	14.30 (5.20)	27.70 (7.94)	23.00 (12.40)
Glycogen	66.52 (10.83)	56.11 (10.51)	6.10 (2.52)	3.96 (2.78)	42.89 (25.61)
Glucose-6P	1.18 (0.54)	4.21 (0.86)	0.22 (0.17)	0.25 (0.23)	1.26 (0.34)
Glucose	0.20 (0.04)	2.35 (0.57)	0.12 (0.09)	0.34 (0.37)	0.37 (0.29)
Fructose-6P	0.23 (0.09)	1.09 (0.20)	0.06 (0.03)	0.08 (0.08)	0.28 (0.08)
ATP	5.61 (0.50)	4.25 (0.68)	3.00 (1.20)	2.06 (1.29)	4.93 (0.70)
ADP	1.24 (0.07)	1.36 (0.18)	0.95 (0.06)	0.99 (0.38)	1.17 (0.20)
AMP	0.04 (0.03)	0.04 (0.01)	0.40 (0.42)	0.17 (0.09)	0.78 (0.09)
IMP	<0.01	0.42 (0.39)	0.40 (0.34)	0.86 (0.35)	0.39 (0.46)
Inosine	<0.02	⩽0.09	0.31 (0.49)	1.09 (0.79)	0.12 (0.07)
Hypoxanthine	⩽0.02	⩽0.08	0.08 (0.01)	0.16 (0.09)	0.04 (0.01)

Table 2. Contents of metabolites 42 h post mortem.

pH	5.55 (0.12)	5.68 (0.07)	6.56 (0.20)	6.53 (0.24)	6.04 (0.12)
Lactate	93.62 (5.96)	92.03 (5.49)	45.17 (14.97)	52.07 (20.93)	90.49 (7.66)
Glycogen	13.62 (5.53)	20.60 (8.44)	0.22 (0.13)	0.60 (0.93)	9.91 (6.74)
Glucose-6P	12.14 (0.96)	11.76 (1.33)	0.22 (0.21)	0.40 (0.67)	4.78 (1.25)
Glucose	4.57 (0.41)	5.51 (0.46)	0.60 (0.42)	0.45 (0.41)	1.15 (0.99)
Fructose-6P	2.42 (0.22)	2.48 (0.27)	0.05 (0.04)	0.08 (0.09)	0.28 (0.21)
ATP	⩽0.38	<0.14	0.12 (0.05)	⩽0.20	⩽0.70
ADP	0.36 (0.05)	0.53 (0.07)	0.64 (0.14)	0.38 (0.10)	0.52 (0.06)
AMP	0.29 (0.09)[1]	0.18 (0.10)	0.35 (0.06)	⩽0.40	⩽0.40
IMP	4.45 (0.17)	3.92 (0.34)	2.55 (0.54)	2.40 (1.00)	4.33 (0.21)
Inosine	0.31 (0.08)	0.54 (0.08)	1.28 (0.62)	1.76 (1.19)	0.34 (0.11)
Hypoxanthine	0.08 (0.03)	0.22 (0.09)	0.43 (0.24)	0.56 (0.22)	0.10 (0.03)

1) n = 7

The discussion below is mainly based on comparisons of two or more of the different groups.

Normal - unstimulated versus stimulated

Data in Table 1, 1 h after slaughter, clearly show the fast pH decline caused by stimulation, resulting in higher amounts of lactate, G6P, glucose and F6P, (all P <0.001), compared to the control group. The glycogen level, in the stimulated group at 1 h, is not significantly different from, but slightly lower than the control group. Data show that ultimate pH was slightly higher (P <0.05) in the stimulated group compared to the control group. None of the glycolytic metabolites were significantly different in the two groups at 42 h after slaughter, except the content of glucose which was higher (P <0.001) in the stimulated group.

Concerning ATP degradation, data show lower content of ATP after 1 h (P <0.001), and higher content of IMP in the stimulated group. The contents of ADP and AMP were not significantly different between the groups, and the contents of inosine and hypoxanthine were very low.

Bendall (1976) showed that 50% of the ATP was depleted in less than 1 h in HVS LD-muscle (at a depth of 5 cm), and that 10 h was required for unstimulated muscle. Tarrant and Mothersill (1977) and Bendall (1976) found an initial level of ATP in normal LD muscle of about 6 µmol/g. The level of 4.25 µmol/g obtained in this study of the stimulated group thus shows a somewhat slower ATP depletion. At 42 h (Table 2) the contents of ATP were low in both groups, ADP higher (P <0.001) in the unstimulated group and AMP lower (P <0.05). The content of IMP was lower (P <0.01), and inosine and hypoxanthine higher (P <0.001) in the stimulated group.

DFD - unstimulated versus stimulated

Data show no significant difference in pH after 1 h although the content of lactate was higher in the stimulated group (P <0.05). No significant differences in contents of the other glycolytic metabolites were found after 1 h. After 42 h, pH and the levels of all glycolytic metabolites showed no significant differences between the two groups.

Concerning ATP degradation, data after 1 h show lower content of ATP, higher contents of IMP, inosine and hypoxanthine in the stimulated group. After 42 h lower IMP, higher inosine and hypoxanthine contents were found after stimulation although none of these values (1 and 42 h) were significantly different between the two groups. No significant differences in contents of ADP and AMP after 1 h were found but the ADP value after 42 h was higher in the unstimulated group (P <0.05).

Normal versus DFD - without and with stimulation

Comparing normal and DFD samples, after 1 h, without stimulation, there were differences only in levels of glycogen ($P < 0.001$), G6P ($P < 0.01$) and F6P ($P < 0.01$) all three being lower in DFD samples. Stimulation, however, resulted in all glycolytic metabolites being lower in the DFD group, lactate ($P < 0.01$), glycogen ($P < 0.001$), G6P ($P < 0.001$), glucose ($P < 0.001$), and F6P ($P < 0.001$).
After 42 h the contents of all glycolytic metabolites were lower in DFD than in normal samples ($P < 0.001$) both in the stimulated and the unstimulated groups. Thus the results show that there is a clear-cut difference in metabolic pattern between normal and DFD muscle irrespective of stimulation.
According to Hamm et al. (1973) glycogen, glucose, G6P, F6P and lactate make up more than 95% of the total glycolytic metabolites in muscle at all times post mortem. Based on this assumption we calculated the recovery of metabolites from glycogen lost between 1 and 42 h post mortem (expressed as glucose equivalents). The recovery for the unstimulated normal group was found to be 117.6 $\pm$ 14.4% and for the stimulated normal group 99.3 $\pm$ 16.0% ($P < 0.05$). The levels are higher than in the studies by Hamm et al. (1973) and Hamm (1977) who in unstimulated bovine muscle with high levels of glycogen 1 h post mortem found around 90% recovery from glycogen, although there were some large variations between the samples. The same calculation, as above, for the DFD groups show much higher formation of lactate plus intermediates than the depletion of glycogen indicates (compared to normal groups). This is the case for all samples in both the unstimulated and stimulated DFD groups. The results indicate that there could be a difference between normal and DFD muscle in lactate formation. Lactate in DFD has probably been formed from some other source not investigated here.
In unstimulated DFD muscle Fischer and Hamm (1980) have shown that the content of ATP, 30 min after slaughter, was lower compared to a normal group, due to exhaustion. Rapid ATP depletion was also shown to result in lower IMP and higher inosine and hypoxanthine 42 h post mortem in unstimulated DFD pigs compared to a normal group. (Potthast and Hamm 1976). Hamm (1977) did also show that a maximum of IMP is reached at about 48 h in normal muscle. Data in our study show that when comparing unstimulated normal group to unstimulated DFD group, after 1 h, the contents of ATP and ADP were both higher ($P < 0.001$) and AMP lower ($P < 0.05$). After 42 h ADP, inosine and hypoxanthine were lower ($P < 0.001$, $P < 0.001$, $P < 0.01$), IMP higher ($P < 0.001$) but no significant difference was found for AMP. Stimulation, when comparing the normal group to DFD, resulted in higher contents of ATP ($P < 0.01$), ADP ($P < 0.05$) and lower contents of AMP ($P < 0.01$) after 1 h together with higher contents of ADP ($P < 0.05$), IMP ($P < 0.01$) and lower contents of inosine ($P < 0.05$) and hypoxanthine ($P < 0.01$) after 42 h. Our data thus clearly show that exhaustion result in lower contents of ATP and higher contents of IMP after 1 h and also more rapid degradation of IMP to inosine and hypoxanthine after 42 h. Stimulation increases the extent of ATP degradation towards hypoxanthine in both the normal and DFD groups, but is still fastest in both DFD groups, which results in the following order for the 4 groups: stimulated DFD > unstimulated DFD > stimulated normal > unstimulated normal.

Unstimulated medium group

After 42 h the contents of lactate, glycogen, IMP, inosine and hypoxanthine were not significantly different from the unstimulated normal group, but comparing to the unstimulated DFD group lactate ($P < 0.01$), glycogen ($P < 0.05$) and IMP ($P < 0.001$) was higher, inosine ($P < 0.05$) and hypoxanthine ($P < 0.05$) lower. However, glucose and F6P were lower and ADP was higher (all $P < 0.001$) than the normal group, but not significantly different from the DFD group. The level of G6P was found to be in between the unstimulated normal and DFD groups, different from both groups ($P < 0.001$)). Thus the medium group could be characterized as normal with respect to some metabolites and as DFD with respect to others. There are even metabolites with levels in between.

Stimulated medium group

The 4 samples in the stimulated medium group, did not show the same uniform pattern of levels of metabolites as the 4 samples in the unstimulated group. They are therefore not included in the tables and will be discussed individually as samples A, B, C and D. Sample A, with an ultimate pH of 5.90, was, after 42 h, found to have levels as the stimulated normal group of lactate (92.08), IMP (3.50), inosine (0.44) and hypoxanthine (0.10) and as the stimulated DFD group of glucose (0.63), F6P (0.17), ADP (0.35) but in between of glycogen (8.33) and G6P (5.57). For sample B, with an ultimate pH of 6.18, the contents of lactate (68.83), glykogen (0.36), G6P (0.96), glucose (1.38), F6P (0.32) and ADP (0.41) at 42 h all were at the same levels as the stimulated DFD group. IMP (3.70), inosine (0.54) and hypoxanthine (0.20) were more close to the levels of stimulated normal group. Sample A could thus be characterized as being in a medium group, but sample B was very similar to a DFD sample.
Sample C showed a fast pH decline after 1 h (pH 5.80), which was lower than any sample in the stimulated normal group and ultimate pH was found to be 5.54. pH was also measured at 3 and 26 h after slaughter (Fjelkner-Modig och Rudérus 1981) which confirms the low level of pH. ATP depletion was at the same level as stimulated normal group. After 42 h sample C had levels of lactate (91.98), glycogen (16.48), IMP (3.40), inosine (0.51) and hypoxanthine (0.13) as the stimulated normal group. However, low levels of G6P (5.49), glucose (0.29), F6P (0.08) and ADP (0.35) were found, which are at the same level as the

stimulated DFD group. Finally sample D, after 1 h showed a fast pH decline (pH, 5.90), high contents of lactate (79.88) and fast ATP degradation (ATP 0.43, IMP 3.5). After 42 h ultimate pH was found to be 5.82. Levels of glycogen (14.18) inosine (0.59), hypoxanthine (0.22) were the same as the stimulated normal group, while glucose (0.77), F6P (0.15), ADP (0.35) and IMP (2.80) were at the same levels as stimulated DFD and levels of lactate (79.81), G6P (3.75) were found to be in between. With respect to levels of metabolites at ultimate pH samples C and D both could be characterized as medium samples.
Sample C and especially sample D, both with rapid glycolysis, could probably be defined as PSE, as described by Hamm and Hoof (1970) and Fischer and Hamm (1980). Wateriness was found to be higher in both samples compared to the control group (Fjelkner-Modig and Rudérus 1981). No sample in the stimulated normal group showed such a fast ATP degradation as sample D. Bendall (1980) discusses the possibility of PSE due to stimulation, but did not find any differences in drip between stimulated and control normal groups. The results obtained here indicate that stressed animals which still have rather high contents of glycogen and ATP before slaughter, probably could be prone to rapid glycolysis and ATP depletion and thus may show properties as PSE when electrically stimulated.

CONCULSIONS

Results of this investigation show

- that stimulation does not affect the incidence of DFD
- that stimulation speeds up glycolysis, but that final contents of most metabolites are at the same levels as for unstimulated muscle. This is true both for normal, DFD and medium groups.
- that the medium groups with respect to different metabolites could be characterized as in some part normal and in some other as DFD.
- that in the DFD groups, both stimulated and unstimulated, the formation of lactate plus intermediates are much higher than the depletion of glycogen indicates.
- that concerning the extent of ATP degradation towards hypoxanthine the following order for the different groups were found to be: stimulated DFD > unstimulated DFD > stimulated normal > unstimulated normal.
- that, in stressed animals, muscles which still have rather high levels of glycogen and ATP before slaughter, when electrically stimulated could be more prone to show PSE characteristics than unstimulated muscles.
- that since the levels of glucose, G6P and F6P are important for keeping quality, flavour and browning during frying the lower contents found in DFD and medium groups (compared to normal) could be of large importance. Stimulation has probably no effect in this respect.
- that since the levels of IMP found were much lower in DFD (compared to medium and normal groups), irrespective of stimulation, this could be of importance for flavour.

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