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ON-LINE IDENTIFICATION OF DFD BY LOCAL ELECTRICAL STIMULATION AND pH MEASUREMENT ON BOVINE CARCASSES

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INTRODUCTION

Since the mid-eighties use of electrical stimulation of beef carcasses has increased in European slaughterhouses. In Denmark low voltage stimulation of whole carcasses is today practised by a current pathway from nose to earth via the shackle. A peak voltage up to 85V and 14 impulses/s. are normally used in production facilities with a stimulation time of 25 to 40 seconds per carcass (Buchter, 1984).

Recently, Braathen (1990) has proposed an additional local electrical stimulation (LES) in the loin in order to accelerate the rigor mortis process within the locally stimulated area.

Carcasses with high ultimate pH (pH>6.2) occur in approximately 5% of young bulls and 2% of dairy cows slaughtered in Denmark (Hald and Jensen, 1992).

Selection of DFD carcasses is a well known technique by measuring pH in the LD 24 to 26 hours after slaughter. Some plant managers claim that rigor mortis can be seen in the hot carcass by observing contraction of the loin and stiffening of the foreleg. Using these observations apparently some of the potential DFD carcasses can be selected.

Low voltage electrical stimulation of the whole carcass will accelerate the onset of rigor mortis, and pH may be measured three to five hours after slaughter on beef carcasses which are still warm. Hereby detection of potential DFD carcasses may be possible, and selection of the carcasses can take place in the chilling room.

The objective of this experiment was to test Braathen's concept for on line identification of DFD carcasses. It was the aim to detect potential DFD carcasses on the slaughterline and hereby select the hot carcasses prior to entry into the chilling room. The method has been used for some years at slaughterhouses in Finland (Röpelinen, 1990) and recently also in Norway. In all plants the method is used to avoid DFD carcasses before hot boning.

MATERIALS AND METHODS

The concept used in the experiments is shown in Figure 1.

LES was performed in the left loin at the 10^{th}-13^{th} rib immediately after the dehiding process. The equipment used is shown in Figure 2.

In early experiments carcass stimulation was omitted but it became evident that the standard deviation of pH measurements became substantial (Hald & Klastrup, 1992). Therefore, the following and conclusive experiments involved both carcass stimulation and LES.

LES was performed approximately 25 minutes after stunning. Stimulation time was varied between one and 2.5 minutes

using DC impulses of 20Hz. The onset of rigor mortis in the stimulated area of the loin was checked by pH measuring approximately 25 minutes later.

pH measurement was performed with an Ingold LDT 406 M3 glass electrode which was inserted in the loin twice in the locally stimulated area within a distance of 4 to 8cm. When the glass electrode was inserted in areas with too much fat in the muscle, the reading was clearly disturbed and would be close to pH7. In these cases the insertion was repeated.

A new Ion Sensitive Field Effect Transistor (ISFET) solid state electrode was tested using Sentron 2001 pH-meter (Kress Rogers, 1991). The reading with the 125mm long and 8mm thick solid state electrode was made at the same points as with the glass electrode, and pH was noted as an average of the two readings.

A galvanic effect was observed at the anode when using an electrode made of stainless steel (AI Si 316) for the local stimulation. Discoloration occurred in the meat within a range of about 2cm from the positive electrode.

A grey metmyoglobin discoloration was observed on a slice of LD 40 to 80 minutes after exposure to oxygen in normal atmosphere. Ten to 24 hours later the discoloration was very intensive and would cause rejection when placed in sales counter. A solution to this problem was found by replacing the stainless steel electrode by an electrode of titanium covered with a thin layer of platinium (Hald, 1993).

Three different stimulation times were tested under practical conditions in a meat plant (Table 1).

The relationship between pH measurement and time was modelled for 879 carcasses (experiment F and E, 493 young bulls and 386 cows) measured at approximately 25, 140 and 1200 min. after LES. EUROP-fatness classification was 1-4 with 58% classified as 3. Data are shown in Figure 4.

A mixed model was used for this analysis (procedure MIXED, SAS, 1992).

Where $y_i'=(y_1,y_2,y_3)$ represents pH-measurement of the i'th carcass. y_i, i=1,2,..,879, are assumed independent distributed, and with $y=(y_i,i=1,2,..,879)$ the model was:

$$y = X\beta + \epsilon$$

Where β is an unknown vector of fixed-effects parameters with known design matrix X and ϵ is an unknown random error vector of independent random variables. The expectations of y and ϵ are respectively $E(y) = X\beta$ and $E(\epsilon)=0$. The variance of ϵ is R, where R is block-diagonal. The diagonal elements of R are identical unstructured 3x3 matrices. Similar covariance structure was assumed for all y_i, i=1,2,..,879, although time of the three pH-measurements are not quite identical.

Model parameters

Main effects and interactions:
Category (Young bull/Cow)
LES-time (1 min/2.5 min);
EUROP-fatness (1..4)
Covariates:
Carcass weight (Mean: 263 kg & standard deviation 48 kg)
with separate slope within Category
t = Time of pH-measurement (app. 25, 140 & 1200 min)
$\sqrt{t}$ and $\sqrt[3]{t}$ with seperate slopes within LES-time

Covariance parameter estimates

Estimates of the diagonal-elements in R are given by:

0.02107919	0.00325842	0.00121695
	0.02783932	0.00457001
		0.00612579

χ^2-test for necessity of modelling the covariance structure was significant (χ^2= 592.7, Df=5, p=0.000). Akaike's Information Criterion (AIC) value was 1657.6. Modelled covariance structure was furthermore justified by comparison with $R = \sigma^2 I$ and a AIC=1375.4.

RESULTS AND DISCUSSION

Accuracy of pH measurements

In all three experiments the standard deviation of repeated measurements was lowest when measuring pH 24 hours after LES.

It seems difficult to obtain an accuracy (SD) better than 0.15 pH units in warm carcasses. Factors affecting the accuracy include:

- Equipment (electrode, cleaning and adjustment)
- Operation (correct handling)
- Insertion of electrode
- Variation of pH within the loin

The new ISFET solid state electrode was resistant and constructed specially for operation under slaughterline conditions. Solid state and glass electrodes were compared by reading in the same point and in the same depth of the loin. Measurements were carried out twice, and the mean was calculated.

Apparently, the application of a solid state electrode will give as reliable and precise results as the glass electrode. Unfortunately, the ISFET pH-meter did not work satisfactorily in the chilling room, so no comparison could be made on chilled carcasses.

Performance of on line identification of DFD carcasses on the slaughterline

Optimal results of the experiment would be 100% recognition of DFD carcasses at the first measurement (pH-1). Assuming that all the experiments (total) represent practical variation of LES approximately 80% of all carcasses could be sorted out as normal on the slaughterline, and two hours later it was possible to identify the remaining DFD carcasses as shown below (criteria for selection and results are described in appendix A):

Pre-selection (pH-1)	low pH	medium pH	high pH
	79.3%	18.2%	2.5%

Final pH (pH-24)	normal pH	interm. pH	high pH
	94.5%	2.8%	2.7%

The results show that about 20% of all carcasses must be measured twice in order to be able to identify all high pH carcasses. The second pH measurement may be carried out two hours later at the earliest, or alternatively on the day after

slaughter. However, preliminary results from a different experiment indicate promising results when measuring as early as five and 30 minutes after LES (Möller, 1993). LES was performed in three minutes with 600 to 700m.amp.

Meat quality

During the initial tests the meat quality was examined by measurement of shear force, meat colour and drip loss by comparison of LES and non-LES carcass sides (Hald, 1991). Main results were:

- Significant difference in pH-1 between LES loin compared to control side (***)
- A mean difference of 0.42 pH units between LES loin compared to control side
- Slightly improved shear force in LES loin (**)
- Slightly darker colour (*) in LES loin. The difference was not visually apparent
- No difference in drip loss in vacuum packs after 10 days

Relationship between time and pH-development

In table 4 significance levels of SS type III F-test are shown. Two of the interactions are insignificant implying a potential model reduction. However in a model without assuming covariance structure, these effects would have been significant. A model using separate slopes within time of pH-measurement within each level of category was not significantly better.

The residual standard deviation of this model was 0.135.

Ignoring the factors category and EUROP-fatness the following parameter estimates are achieved, Table 5.

Predicted values for a mean carcass weight were estimated from parameters in Table 5 and shown in Figure 5. Time of local stimulation affected pH-development in a similar way, but one minute accelerated pH-drop slightly more than 2.5 minutes. A remarkable drop in pH is observed followed by a small increase before final pH is established.

CONCLUSION

- No inconvenience was found for the slaughterline operator using LES equipment.
- Meat quality was not affected by LES of the loin.
- Electrode for LES must be of a suitable material to avoid discoloration.
- 80% of carcasses could be segregated as normal (pH<5.8) by pH measurement on the slaughterline.
- Two hours later the rest of the carcasses could be segregated into groups of elevated pH (5.8<pH<6.2) or DFD (pH>6.2).
- Standard deviation for pH-1 measurement (on the slaughterline) was 0.15 using glass electrode and 0.18 using solid state ISFET electrode.
- Standard deviation for pH24 measurement was 0.07 using glass electrode.
- Relationship between pH-development and time was modelled with a standard deviation of 0.138.
- LES for one and 2.5 minutes affected pH-development in a similar way, but 1 minute accelerated pH-drop slightly more.

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Appendix A. Effect of stimulation time in three experiments. Results by simple sorting. Numbers shown as row %.

A	Final pH	Normal pH			Intermediate pH			High pH		
B	Pre-selection	Low	Med	High	Low	Med	High	Low	Med	High
C	1 min N=422	78.4	14.5	0.7	1.0	2.6	0.5	0	1.0	1.4
C	2½min N=457	70.0	21.2	1.1	2.0	1.1	0	0.2	1.3	3.0
C	1+1mi N=643	83.4	12.6	0.3	0.8	1.2	0	0	0.7	0.9
D	Total N= 1552	78.1	15.7	0.6	1.1	1.5	0.2	0	1.0	1.7

A Final pH (pH-24) divided into normal, intermediate and high pH

Normal pH	pH<5.8
Intermediate pH	5.8<pH<6.2
High pH	pH>6.2

B Pre-selection pH-1, subdivision into

Low	5.5<pH<6.0
Medium	6.0<pH<6.4
High	pH>6.4

C LES in minutes

D Total = total of the three experiments, assuming that the three experiments represent the variation of LES that might occur in practice.

Table 1. Number of carcasses, carcass weight and time of measurement in trials E, F, G.

Exp. (LES, min.)	Number of carcasses by category; Carcass weight & SD,kg		pH measurements: pH-1[1] pH-2[2] pH-24[3]	Ave.time after LES: Mean	Ave.time after LES: S.D
E (2½)	Young bulls:	237	pH-1	27	15
	Cows:	220	pH-2	144	49
	Carcass weight:	264.3	pH-24	1190	140
	SD:	47.3			
F (1)	Young bulls:	256	pH-1	24	14
	Cows:	166	pH-2	139	47
	Carcass weight:	262.6	pH-24	1206	127
	SD:	48.8			
G (1+1)	Young bulls:	449	pH-1	28	21
	Cows:	231	pH-24	1150	143
	Carcass weight:	252.2			
	SD:	45.0			

1 pH-1 is the first pH-measurement, performed immediately after weighing
2 pH-2 is the second pH-measurement, performed in the chilling room 2-3 hours after LES
3 pH-24 is the final pH, performed on chilled carcasses in the chilling room before boning

Table 2. Accuracy of repeated pH-measurements with glass electrode (first ÷ second measurement).

pH-measurement	Experiment	LES-time, min.	Number of carcasses	pH measurement	
				Average difference	Standard deviation
pH-1	F	1	422	0.002	0.101
	E	2 1/2	457	-0.003	0.161
	G	1 + 1	574	0.013	0.156
pH-2	F	1	422	-0.009	0.168
	E	2 1/2	456	-0.110	0.301
	G	-	-	-	-
pH-24	F	1	422	-0.008	0.052
	E	2 1/2	457	-0.007	0.069
	G	1 + 1	678	-0.002	0.054

Table 3. Comparison of solid state and glass electrode accuracy (first ÷ second measurement and ISFET ÷ glass electrode*).

	Number of carcasses	Minimum	Maximum	Mean	Standard deviation
pH-1 ISFET	317	-0.85	0.60	0.0170	0.181
pH-1 Glass	574	-0.54	0.69	0.0130	0.155
ISFET ÷ glass	218	-0.46	0.43	0.10	0.13

* Repeated measurements of both electrodes

Table 4. ANOVA-table of pH - time model .

Tests of fixed effects			
Source	DF	Type III F	Pr > F
LES time	1	13.80	0.0002
Category	1	6.58	0.0104
EUROP-fatness	3	12.98	0.0000
Category*LES time	1	9.43	0.0022
EUROP-fatness*LES time	3	0.78	0.5025
Category*EUROP-fatness	3	2.46	0.610
Carcass weight*Category	1	13.94	0.0002
Carcass weight	1	5.69	0.0171
t = Time of pH-measurement	1	66.62	0.0000
t*LES	1	15.80	0.0001
$\sqrt{t}$	1	246.96	0.0000
$\sqrt{t}$*LES	1	18.01	0.0000
$\sqrt[3]{t}$	1	421.24	0.0000
$\sqrt[3]{t}$*LES	1	18.01	0.0000

Table 5. Parameter estimates for main fixed effects.

Solution for fixed effects		
Parameter	Estimate	Standard error
Intercept	7.05730	0.07694
LES time 1.0 min.	0.40890	0.11206
LES time 2.5 min.	0.00000	
Carcass weight	-0.00008	0.00005
t(LES time 1.0)	-0.00126	0.00016
t(LES time 2.5)	-0.00041	0.00015
√t(LES-time 1.0)	0.24839	0.01901
√t(LES-time 2.5)	0.14185	0.01748
^{3}√t(LES-time 1.0)	-1.06745	0.06503
^{3}√t(LES-time 2.5)	-0.70157	0.06020