EFFECT OF BREED, ELECTRICAL STIMULATION AND MUSCLE ON RATE OF PH DECLINE AND ULTIMATE PH IN CATTLE POST-MORTEM

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Background

Glycolysis and the associated pH decline are of primary importance to quality during meat processing. The rate of pH decline in a carcass relative to the chilling rate affects tenderness, the water binding capacity of meat, meat colour, and meat colour stability. Electrical stimulation is used to control glycolytic rate, and in recent years electrical inputs have been optimised for particular slaughter operations. Despite these efforts there is anecdotal "industry" evidence suggesting that considerable variation still exists.

Glycolytic rate following slaughter can be influenced by a number of factors. Many of these are associated with temperature, including variations in thermal inertia due to carcass size and fat depth, and different chilling practices (Jolley *et al.* 1980-81). However, the metabolic characteristics of the muscle itself may also have a role to play. Skeletal muscle consists of several different fibre types, the two main groups being type I (slow), and type II (fast) fibres. Type II fibres are further classified as type IIA which are fast oxidative fibres, and type IIB which are fast glycolytic fibres (Conlee *et al.* 1978). Glycolytic rates are greater in type IIB fibres than in type I or IIA, thus muscles with greater proportions of type IIB fibres display greater glycolytic rates in living tissue (Lawrie 1992). Factors that affect the predominance of these fibre types within muscles, such as genotype may lead to variation in glycolytic rate between animals. Some breeds are noted for greater proportions of type IIB fibres (ie Piedmontese), thus variation in fibre type both between muscles and between animals may affect glycolytic rate.

Objectives

To examine the impact of breed and muscle type and the interaction of electrical stimulation on glycolytic rate in cattle post mortem.

Methods

Animals slaughter and measurements

Twenty four, 9 month old steers (13) and heifers (11) (average liveweight 238kg) previously maintained on a green kikuyu, paspalum, and rhodes grass pasture, were slaughtered after at a commercial abattoir after 1.5 hours transport and 15 hours lairage. There were 8 Wagyu, 8 Angus, and 8 Piedmontese, all first crosses with Hereford. 50 min following slaughter one side of each dressed carcass received high voltage electrical stimulation for 1 min at 830V. No other electrical inputs were applied. Muscle samples, temperature and pH readings were taken from the *m. semimembranosus* (SM) and *m. semitendinosus* (ST) of both the stimulated and non-stimulated sides of the carcass immediately following stimulation, and 1, 2, 4, 6, 7, and 24 h post stimulation. Muscle samples were immediately frozen in liquid nitrogen. Muscle glycogen and lactate concentration was determined for all muscle samples taken, according to the enzymatic methods of Kunst *et al* (1983), and Marbach and Weil (1967). Glycogen concentration represents the sum of glycogen plus free glucose. ATPase activities were determined for the samples taken immediately following stimulation, using a continuous fluorimetric method (Takashi and Putnam, 1979). pH measurements were taken using an Ionode glass pH probe. The pH reading at 24 h was taken as ultimate pH (pHu).

Calculations

All pH values were adjusted to 25°C, using the formula described by Briskey & Wismer-Pedersen (1961):

$$pH_{adj} = pH_{unadj} + (Temp_{act} - 25) * 0.01$$

Due to differential rates of temperature decline in the SM and ST, pH values were adjusted using an exponential equation described by Bendall (1978), which adjusts pH by a rate constant to assume glycolysis occurring at a constant temperature of 25°C:

$$pH_{rate\ adj} = pH_{unadj} * (\exp(16.145\text{-}4811.2/Temp_{act}))$$

where $Temp_{act}$ is absolute temperature (K). pH and temperature as a function of time were analysed using the exponential function described by Bruce *et al* (2001):

$$y_t = a_u + (a_i - a_u)e^{-akt}$$

where y_t is pH, or temperature, as a function of time, a_u is ultimate pH, or temperature, a_i is initial pH, or temperature, a_k is the rate of pH or temperature fall and t is time. From these functions an exponential rate constant (pHk) was derived for each side of each carcass. pHk values for the stimulated sides did not include a pre-stimulation pH value. pH fall during stimulation (ΔpH) was calculated for each carcass based on the difference in pH between the stimulated and non-stimulated sides immediately after stimulation was applied.

Statistical analyses

PROC MIXED (SAS) was used to examine the effect of breed, muscle type and stimulation, and interactions between these terms, with animal as a random effect, on pHk, pHu, and ATPase activities. ΔpH was analysed in the same way, without stimulation in the model. Muscle glycogen and lactate concentrations and ATPase activities were included as covariates in all analyses (except where they were the dependant variable). Non-significant terms ($P > 0.05$) were sequentially deleted from the models.

Results and Discussion

ATPase activities

ATPase activities differed between muscles as expected, with higher Ca^{2+}-dependent myofibrillar ATPase and lower total Mg^{2+}-dependent ATPase activities in the faster anaerobic ST than the more aerobic SM (Table 1). Following stimulation the Ca^{2+}-dependent myofibrillar ATPase activity increased by 20-30%, and the total Mg^{2+}-dependent ATPase actvity decreased by 25% in both muscles (Table 1). Ca^{2+}-dependent myofibrillar ATPase was also affected by breed, with Piedmontese and Angus cattle (0.16±.006 across both SM and ST) having higher activities than Wagyu (0.14±.003; $P<0.05$). Stimulation and muscle did not affect the Sarcoplasmic reticulum (SR) Ca^{2+}-dependent ATPase activity, although breed did have an impact. SR Ca^{2+}-dependent ATPase activities were higher in the Piedmontese (0.034±.003

across both SM and ST) than either Angus or Wagyu (0.028±.003; P<0.05). These results highlight the fast glycolytic nature of the Piedmontese muscle in contrast to the Angus and Wagyu breeds.

ΔpH

Stimulation caused a significant immediate drop in pH (ΔpH) in the SM yet no marked change in the ST (Table 1). Muscle lactate concentration affected this response, as a 40mM rise in lactate concentration (concentrations ranged from 3 – 48mM) would cause and estimated reduction in ΔpH of about 0.5 of a pH unit. The effect of lactate may partially account for why the ΔpH in the ST showed minimal response. Associated with the higher glycolytic rate in the ST were higher muscle lactate concentrations at the time of stimulation (ST, 37±3.1mM; SM, 28±3.1mM). ΔpH was also affected by breed, with a larger drop in the Piedmontese (ΔpH 0.2±.07 across both SM and ST) than either the Angus or Wagyu (ΔpH -0.05±.07 across both SM and ST; P<0.01) cattle. However, given the effect of breed on the Ca^{2+}-dependent myofibrilar ATPase (higher in Piedmontese and Angus) and the SR Ca^{2+}-dependent ATPase (higher in Piedmontese), and that these ATPases were interchangeable with breed in the ΔpH model, having a positive effect on ΔpH, it suggests that the breed effect was largely driven by these two ATPase activities.

pHk

In non-stimulated carcasses the rate constant for pH decline (pHk) in the ST was 70% higher than in the SM (P<0.01) indicating a faster glycolytic rate in the ST (Table 1). However, in response to stimulation the rate constant for the SM increased by about 50% (P<0.01). The ST remained unchanged, thus the two muscles had similar glycolytic rates following stimulation (Table 1). In contrast to ΔpH neither glycogen and lactate or the ATPases were significant covariates for the rate of decline. Breed also had no effect, possibly reflecting the lack of ATPase responses.

pHu

Ultimate pH (pHu) varied only marginally between muscles (Table 1), to be expected given that glycogen concentrations for most animals and in both muscles were adequate to reach a pHu of 5.5 or below (ie 60μmol/g for the ST and 78μmol/g for the SM) (Warriss 1990). Stimulation increased pHu in the ST (P<0.05), yet once again this effect was small (0.07 pH units) (Table 1).

Conclusion

Breed had significant effects on ATPase activities, suggesting associated differences in metabolic and contractile properties of muscle. These differences played key roles in ΔpH response, thus breed and ATPase activities were significant predictors of ΔpH. However they did not play an important role in determining pHk. Thus some other biochemical property of muscle which differs significantly between the SM and ST needs to be identified to understand the major determinants of pHk.

Pertinent literature

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Table 1. Predicted pH change through stimulation (ΔpH), pH decline exponential rate constant (pHk) ultimate pH (pHu) and ATPase activities after adjustment for a random animal effect. Values are means ± SE.

	Control		Electrical Stimulation		Significance of Effects		
	SM	ST	SM	ST	Muscle	Stimulation	Interaction
ΔpH ^{zy}			0.11±0.05	-0.05±0.05	*	n.a.	n.a.
pHk	4.60±.41^{a}	7.69±.41^{c}	6.60±.41^{b}	7.00±.41^{bc}	**	**	**
pHu ^{yx}	5.39±.02^{b}	5.29±.02^{a}	5.40±.02^{b}	5.36±.02^{b}	**	*	*
Ca^{2+}-dependent myofibrillar ATPase^{z} (Units/gm protein.min)	0.13±.006^{a}	0.17±.006^{b}	0.15±.006^{c}	0.18±.006^{d}	**	**	ns
Sarcoplasmic reticulum Ca^{2+}-dependent ATPase (Units/gm protein.min)	0.030±.003^{b}	0.033±.003^{b}	0.026±.003^{b}	0.031±.003^{b}	ns	ns	ns
Total Mg^{2+}-dependent. ATPase Mg dep. (Units/gm protein.min)	0.22±.01^{a}	0.17±.01^{b}	0.18±.01^{b}	0.13±.01^{c}	**	**	ns

Values within rows with different superscripts are different at P<0.05. ns - not significant; * - P<0.05; ** - P<0.01; n.a. – not applicable.
Adjustments made for significant covariates: ^{z} Breed; ^{y} Lactate; ^{x} Glycogen.