PREDICTING POST-SLAUGHTER MUSCLE GLYCOGEN CONTENT AND DARK-CUTTING THROUGH CHANGES IN PRE-SLAUGHTER BODY TEMPERATURE OF LAMBS

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***Abstract*— Exposure to pre-slaughter stress may decrease muscle glycogen content, a key element for a suitable low ultimate pH and prevention of dark-cutting meat. The measurement of body temperature is a tool used in animal stress studies to quantify the impact of the specific stressors, particularly those that elicit fear responses. The relationships between body temperature of sheep and post-mortem muscle glycogen concentration was investigated in the present study, using forty cross-bred female lambs. Body temperature was measured with intravaginal loggers inserted into each animal 5 days pre-slaughter, to record body temperature every 3 min over a period of 3 days. Blood samples were collected from each animal at exsanguination for measurement of glucose and lactic acid concentrations. The concentration of glycogen and lactate were determined in samples of *longissimus thoracis* collected at 1 h post-slaughter. A plot of body temperature over time shows a rise in body temperature, from all animals, during events such as mustering, loading onto the truck, during mustering for slaughter, and also, at slaughter. Pearson correlation coefficients were determined between the main temperature increments occurring between farm and slaughter and post-slaughter muscle glycogen and lactate levels. The initial change in body temperature, in response to mustering on the farm, showed the highest correlation with muscle glycogen ($r=-0.455$; $P<0.01$). Body temperature rise in response to several handling events and ultimate pH in the *semitendinosus* (a stress-sensitive muscle) was also correlated ($r=0.349$; $P>0.05$). Further research is warranted in the study of the relationship between body temperature monitoring and meat quality parameters, in order to understand the complex relationship between animal stress and meat quality.**

***Index Terms*—Glycogen, Temperature, Stress, Dark-Cutting, Ultimate pH**

I. INTRODUCTION

Glycogen concentration at the time of slaughter determines the extent of *post mortem* anaerobic glycolysis in muscle. Inadequate levels of muscle glycogen at slaughter will limit pH decline leading to high ultimate pH meat, which is also known as dark-cutting meat. Dark-cutting meat generally has less acceptable flavour, tenderness, shelf-life and overall acceptability. The storage of muscle glycogen and the rate of *post mortem* pH decline also depend on the muscle fibre composition. Thus, muscles containing a high proportion of oxidative fibres (type I) display faster rates of pH decline compared to those containing a high proportion of glycolytic fibres (type IIb) (Aalhus and Price, 1991).

It is known that different pre-slaughter factors can impact on muscle glycogen storage. In this regard, exposure to pre-slaughter stress can result in reduced muscle glycogen content largely through activation of the sympatho-adrenal response resulting in catecholamine-mediated glycogenolysis (Kuchel, 1991) Several biochemical parameters in blood and muscle have been assayed to objectively quantify animal stress, but there has been no general agreement on a standard measurement . Moreover, it is also known that biochemical changes may depend on the type of stressor. It has been shown that body temperature changes according to the level of stress at several stages of animal handling (Ferguson, 2003). The capacity to continuously log body temperature of freely behaving animals has recently become easy, safe and cost effective (Bluett, Fisher and Waugh, 2000; Lea, Niemeyer, Reed, Fisher and Ferguson, 2008). Therefore, as it is a method that enables measurement in freely behaving animals, it offers advantages over other measures which require the animal to handled and restrained. Therefore it would appear to offer considerable potential in animal stress physiology research.

The aim of the present experiment was to investigate the possible relationship between the body temperature of sheep and *post mortem* muscle glycogen concentration, in order to predict dark-cutting meat.

II. MATERIALS AND METHODS

Animals

Forty cross-bred female lambs (first-cross: Merino x Border Leicester -BL-, and second-cross: Merino x BL x Poll Dorset), approximately 6 months old were used in the present work.

Temperature logging

Temperature loggers (Dallas Thermocron iButton, DS1921 H, Maxim Integrated Products, Sunnyvale, CA, USA) were coupled to progesterone-free ovine intravaginal devices (CIDR Interag, Hamilton, New Zealand) as described by Lea *et al.* (2008). Loggers were inserted intravaginally into each animal, using lubricant, at 5 d pre-slaughter. The temperature data loggers were programmed to start logging on Sunday at 2.00 pm and to end logging on Tuesday at 7.00 pm (day of slaughter), with individual temperatures recorded every 3 min over 3 d. This period, included a period grazing on the farm, mustering prior to transport, yarding for ~6 h pre-transport, loading, transport for ~6 h, unloading, ~12 h in a pen at the abattoir, and assembly prior to slaughter. Temperature loggers were removed at approximately 5 min post-slaughter. Only one data logger was lost during the pre-slaughter phase. Data was downloaded to a PC using the iButton receptor connected to a 1-Wire adapter (Dallas Thermocron, DS1402D-DR8 iButton receptor and DS9490R USB 1-Wire adapter, Maxim Integrated Products, Sunnyvale, CA, USA). Data were displayed and analyzed using the software provided by Dallas Thermocron (Maxim Integrated Products, Sunnyvale, CA, USA).

Samples and pH measurement

Blood samples were collected from each animal at exsanguination into a tube containing heparin as anticoagulant and immediately placed on ice. Samples were centrifuged (3000 x g, 10 min, 4 °C) within 5 h post-slaughter. Plasma was separated and then stored at -20 °C until analysis for glucose and lactic acid concentrations.

Muscle samples were removed from *Longissimus thoracis* (LT) at ~1 h post-slaughter. Samples of LT were immediately frozen in liquid nitrogen until processing for glycogen and lactic acid levels. The pH of the *Semitendinosus* (ST) and LT was taken at 24 h *post mortem*, using a Micrometer pH Vision Model 6007 (Jenco Instruments, San Diego, CA) with a direct pH probe (Ionode Model No. IJ42).

Laboratory Methods

Plasma glucose and lactic acid concentrations were measured using enzymatic commercial kits (Sigma-Aldrich Pty, MO and Randox Labs Ltd., UK, respectively). Muscle glycogen concentration was determined according to Dreiling, Brown, Casale and Kelly (1987). Muscle L-lactic acid level was determined as described by Noll (1985).

Statistical and Data Analysis

Basal temperature for each animal was calculated as the mean of the four lowest temperature values recorded. Main peak increments for specific events were calculated as the difference between the peak temperature and the corresponding basal temperature and were expressed as a percentage of the basal temperature (%). Results obtained are expressed as mean ± standard deviation. Pearson´s correlation coefficients were calculated in order to determine correlations between the different parameters assayed.

III. RESULTS AND DISCUSSION

Mean body temperature recorded during pre-slaughter handling of sheep is shown in **Figure 1**. As can be seen, some events were reflected in several body temperature peaks. Mustering, truck loading and pen change were the events which gave rise to the largest body temperature increments. It is important to remark that those events involving physical exertion as well as human handling were reflected best in temperature logging. The main peak increments are shown in **Table 1**.

Biochemical parameters in blood and muscle are shown in **Table 2**. The Pearson´s correlation coefficients obtained between blood and muscle biochemical parameters, ultimate pH for the LL and ST and major temperature peaks are shown in **Table 3**. As can be seen in **Table 3**, the highest correlation coefficient obtained was between Peak 1 and the muscle glycogen level or glycolytic potential ($r = -0.455$, -0.419 respectively; $P<0.05$ for both). There was also a significant correlation between total temperature increment and muscle glycogen or glycolytic potential ($r=-0.328$, -0.320 respectively; $P<0.05$ for both).

A low and non-significant correlation was found between ultimate pH in the LT, and peak 1, or total increment of temperature ($P>0.05$ for both). There was a significant correlation between the ultimate pH in the ST and the total temperature increment ($r=0.349$; $P<0.05$). None of the carcasses would be defined as dark-cutting (pHu>6.0) in the LT therefore it would be expected that the relationship between ultimate pH in the LT and temperature would not be strong. It is well-known that muscle glycogen is incrementally depleted in response to stress events, and it is not until the muscle glycogen drops below about 45-55 μmoles/g that the ultimate pH of the meat is elevated. The ultimate pH in the ST of several carcasses was elevated (pH>6.0) and the ST is know to be more stress-sensitive then the LT (Pethick et al., 2005).

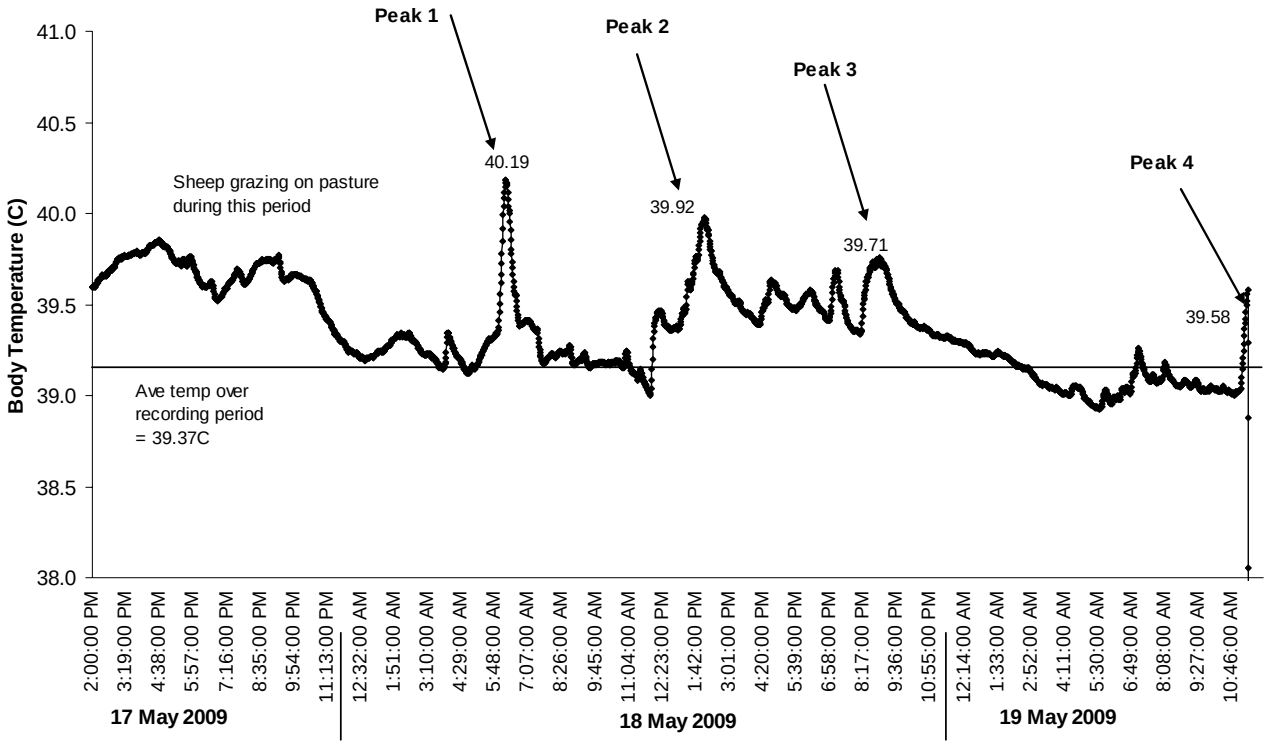

Figure 1. Mean vaginal temperature of sheep (n=39) recorded during pre-slaughter handling from farm to slaughter. Events: **1**, mustering; **2**, truck loading; **3**, pen change at abattoir; **4**, slaughter. The average rise in vaginal temperature (^{o}C) for each event is indicated as well as the average temperature over the entire period.

Table 1. Main peak increments recorded in pre-slaughter handling of sheep

Temperature increment (%)	Mean ± SD
Peak 1 (mustering)	2.67 ± 0.46
Peak 2 (truck loading)	2.17 ± 0.67
Peak 3 (pen change)	1.62 ± 0.63
Peak 4 (slaughter moment)	1.44 ± 0.64
Total Increment	7.91 ± 1.78

Table 2. Biochemical parameters in blood of sheep at exsanguination (slaughter)

Biochemical Parameter	Mean ± SD
Blood glucose (mM)	4.07 ± 0.45
Blood lactate (mM)	1.48 ± 0.69
Muscle glycogen (umol/g wet tissue)	51.34 ± 13.37
Muscle lactate (umol/g wet tissue)	53.28 ± 5.94
Glycolytic potential (umol/g wet tissue)	152.16 ± 34.78

Table 3. Pearson´s correlation coefficients (r) between main peak and total temperature increments recorded and biochemical parameters of blood and muscle and ultimate pH of the *Semitendinosus* (ST) and *Longissimus thoracis* (LT)

Parameter	Peak 1 (mustering)	Total Increment of Temperature
Blood glucose (mM)	- 0.019	- 0.055
Blood lactate (mM)	0.036	0.063
Muscle glycogen (umol/g wet tissue)	- 0.455**	- 0.328*
Muscle lactate (umol/g wet tissue)	0.275	0.123
Glycolytic potential (umol/g wet tissue)	- 0.419**	- 0.320*
Ultimate pH ST	0.238	0.349*
Ultimate pH LT	0.073	0.126

* P<0.05, ** P<0.01

IV. CONCLUSION

Changes in body temperature due to pre-slaughter stress in sheep were correlated with post-slaughter LT muscle glycogen levels at slaughter, and with the ultimate pH of the ST. Thus changes in body temperature pre-slaughter in lambs shows potential for enabling prediction of muscle glycogen levels and dark-cutting post-slaughter.

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