Derivation of the Probability Density Function for Ultimate Muscle pH in Slaughtered Animals

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Abstract We consider the form of the probability density function for the ultimate muscle pH in slaughtered animals. Muscle pH in slaughtered animals is dependent on a biochemical process which forms lactic acid from the breakdown of glycogen stored in the muscle at slaughter. The relationship between glycogen and muscle pH after slaughter is expressed as a pair of coupled differential equations. The solution of this system for the equilibrium muscle pH as time $\to \infty$ gives the form of the probability density for the ultimate muscle pH. When the initial density for the muscle glycogen is normal, the density for the ultimate muscle pH is shown to be approximately a mixed normal density.

Key words: dynamical system; muscle pH; probability density

INTRODUCTION

During the conversion of muscle to meat *post mortem*, the dominant biochemical event is the gradual fall in muscle pH, from approximately 7.2 in the living animal to, typically, 5.5. This gradual acidification of the muscle is the product of the conversion of muscle glycogen to lactic acid, a reaction that regenerates muscle adenosine triphosphate (ATP). Over a *post mortem* period of up to 12 hours in beef muscle, the drop in pH inhibits the resynthesis of ATP, and ATP concentrations decline; *rigor mortis* is defined as complete depletion of ATP (see Bendall, 1973, for a review of the *post mortem* changes in muscle), and the pH of meat at *rigor mortis* as the ultimate pH (pH_u).

The pH_u of meat has important commercial consequences. Compared with meat with a normal pH_u of 5.5, an increase in the pH_u of meat adversely affects colour, flavour, texture and shelf life (Tarrant & Hood, 1981). There are therefore strong commercial justifications for understanding and controlling the causes of elevated pH_u in meat. The pH_u is related to the concentrations of glycogen in muscle at slaughter. Approximately 40 µmol/g of muscle tissue are needed to lower the pH from 7.2 to 5.5. If the concentration of glycogen at slaughter is higher than this value, the residual is not metabolised. When lower than this value, the pH decline is arrested by the lack of substrate (glycogen) and the pH_u is elevated. The relationship between pH_u and initial glycogen concentrations is linear for values of initial glycogen less than 40 µmol/g of muscle, and the slope is proportional to the buffering capacity of the muscle tissue. Muscle glycogen concentrations at slaughter are reduced primarily by stress in the period immediately before slaughter; (Tarrant & Hood, 1981).

Efforts to understand the factors contributing to high pH_u in meat have been difficult because of the nature of the frequency distribution of muscle pH_u in a population of slaughtered animals. Figure 1 shows a typical frequency distribution that exhibits a peaked primary mode with a long upper tail and a suggestion of a secondary mode at high pH_u. While the definition of pH in terms of the hydrogen ion concentration involves logarithms, taking inverse logarithms or any other simple transformation of the pH_u data fails to derive a normal probability density.

Fig 1: Ultimate Muscle pH for Steers Slaughtered at Manawatu Beef Packer's During 1993/94
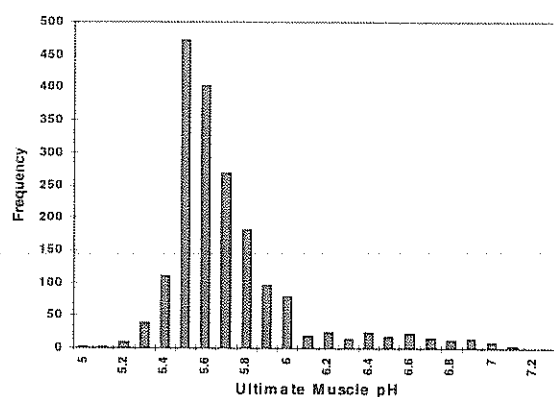
Farmers would like to introduce strategies to breed animals with genetic potential for low ultimate muscle pH. Also meat processors would like to identify farmers who can consistently supply animals for slaughter, which have low pH_u. Both these problems need good estimates of the repeatability of muscle pH_u to be successful. Good estimates of repeatability require a good theoretical understanding of the probability density of the trait in question.

This paper derives a probability density for the ultimate muscle pH_u in slaughtered animals from some basic facts about the breakdown of muscle glycogen to lactic acid in the muscle after slaughter. Using this probability density improved estimates of the repeatability of muscle pH_u between sires (heritability) and between suppliers can be made.

Derivation of the Probability Density for Ultimate Muscle pH in Slaughtered Animals

The biochemical pathway from muscle glycogen to lactic acid (which determines muscle pH_u) involves a complex series of enzymatic reactions (Lehninger, 1978). However, since the end product, lactic acid, is the only aspect of this pathway of interest, the primary dynamics can be described by how the input (muscle glycogen) and the output (lactic acid) change over time. These dynamics can be approximated by:

- The muscle pH and the availability of muscle glycogen determine the rate of change of muscle glycogen over time. As muscle pH falls the breakdown of muscle glycogen falls until, at pH = 5.5, no further muscle glycogen is broken down.
- The rate of change of pH in the muscle also depends on the availability of muscle glycogen and the buffering properties of muscle . Close to muscle pH of 7.2 buffering capacity is high so that a high proportion of hydrogen ions generated by glycolysis is bound and pH falls slowly. Similarly as muscle pH reaches 5.5 glycolysis becomes increasingly inhibited by the pH so that the rate of pH change slows, finally ceasing at a pH of 5.5

These factors can be expressed mathematically in terms of the following pair of differential equations. Here p designates muscle pH and G designates muscle glycogen. The rate of change of both muscle pH and muscle glycogen depends on the same function of the amount of muscle glycogen $y(G)$ expressed as a mass ratio.

$$\frac{dG}{dt} = (5.5 - p)\,y(G)$$

$$k\frac{dp}{dt} = (5.5 - p)(7.2 - p)\,y(G)$$

$$(1)$$

where k is a constant related to the rates of each equation. The units of G are scaled so that $k = 1$.

This system has a set of equilibrium points. Either $y(G) = 0$ and $5.5 \leq p \leq 7.2$, or $p = 5.5$ and $y(G) > 0$. That is, a separate equilibrium point exists for every set of initial conditions. Depending on the initial amount of glycogen and the initial pH the equilibrium state of the pH in the muscle will either be at 5.5, or at a pH of greater than 5.5 depending on the initial amount of glycogen available. The streamlines of the phase plane for this system are shown in Figure 2.

Figure 2: The phase plane for the system of differential equations (1) describing the evolution of muscle pH in meat after slaughter for a normal distribution of initial glycogen levels.
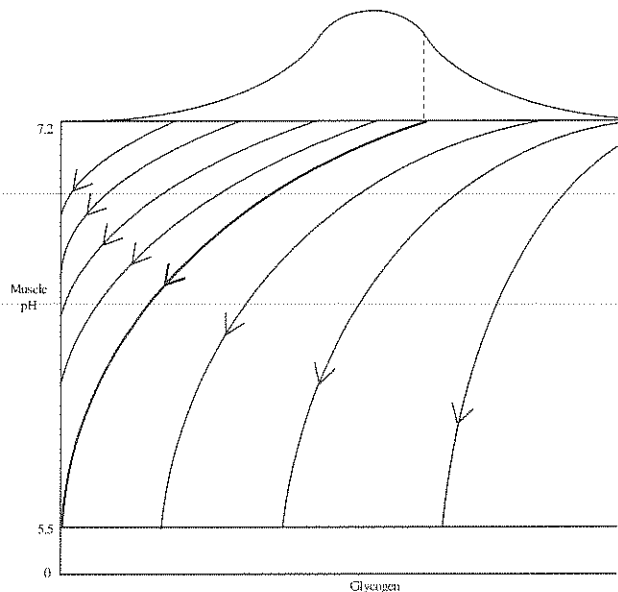
From equations (1) the equation for the streamlines in the pH glycogen phase plane shown in Figure 2 is given by:

$$\frac{dp}{dG} = 7.2 - p$$

This equation has the solution:

$$p = 7.2 - Ce^{-G} \qquad (2)$$

where C is related to the amount of glycogen in the muscle at slaughter, that is at time $t = 0$, and $p(t = 0) = 7.2 - \varepsilon$ where the constant ε allows for the fact that the muscle pH p differs from 7.2 in the live animal, arbitrarily close from below. By writing in (2) the initial condition we get:

$$p = 7.2 - \varepsilon e^{G_0 - G} \qquad (3)$$

This relates the value of G_0 at slaughter to the initial pH value of 7.2.

To achieve a ultimate muscle pH of 5.5 equation (2) shows that for $G \geq 0$:

$$G_0 \geq \ln\left(\frac{1.7}{\varepsilon}\right) = Q$$

Let the probability density of muscle glycogen in a population of live animals be $f(G_0)$ at time $t = 0$. Then from the derivation above:

$$\text{Prob[pH} = 5.5] = \int_Q^\infty f(G_0)dG_0$$

Thus a probability density for the ultimate muscle pH in a slaughtered animal will be:

$$P[p] = \begin{cases} \int_Q^\infty f(G_0)dG_0 \\ \beta\varepsilon e^{G_0} f(G_0) \end{cases}$$

where β is chosen to make P(p) a probability density function and G_0 depends on p. This follows since if $p = F(G_0)$ and G_0 has a probability density function of $f(G_0)$ then p has a probability density function proportional to $F'(G_0).f(G_0)$

The measurement of muscle pH in the slaughtered animal involves instrument error. Assume that this instrument error is a normally distributed random variable with mean zero and variance σ_m^2. Then the observed probability density for the ultimate muscle pH in a slaughtered animal will be the convolution of the instrument error density with the density for the actual ultimate muscle pH:

$$P[p] = N(5.5, \sigma_m^2 \int_Q^\infty f(G_0)dG_0 + $$
$$+ (\beta\varepsilon e^{G_0} f(G_0) * N(0, \sigma_m^2) |_{G_0 = 0}^Q$$

where * is the convolution operator, and the last term indicates that G_0 is truncated at Q.

Let:

$$\alpha = \int_Q^\infty f(G_0)dG_0$$

Then:

$$|P[p] = \alpha N(5.5, \sigma_m^2) +$$
$$+ (1-\alpha)\gamma e^{G_0} f(G_0) * N(0, \sigma_m^2) |_{G_0}^Q \qquad (4)$$

If the pH buffers of 5.5 above and 7.2 below are taken as normally distributed random variables, then the steady states reached by different animals will vary for the same initial amount of glycogen. This variability is factored into the construction of the probability density (4) before the truncation into the mixed density takes place. If an approximation is made by including measurement error as part of the variation associated with the buffer uncertainty then probability density can be expressed as a mixed normal density. Assume that the probability density of muscle glycogen in the live animal is normal, then making the transformation from p to G_0 by $G_0 = \ln(7.2 - p)$:

$$P[p] = \alpha N(\ln(1.7), \sigma_m^2) +$$
$$+ (1-\alpha)N(\mu_{ln}, \sigma_m^2 + \sigma_{lp}^2) \qquad (5)$$

where μ_{ln} is the mean of $\ln(7.2 - p)$ and σ_{lp}^2 is the variance of $\ln(7.2-p)$.

Hosmer(1973) has considered the theoretical issues in obtaining maximum likelihood estimates of the parameters of the mixed normal density and give an iterative algorithm for the solution. Agha and Ibrahim (1984) present a computer program in Algol for these estimates.

The parameters to be estimated are:
μ. The expected value of the amount of muscle glycogen in the population of animals before slaughter.
σ_x^2. The variance of the amount of muscle glycogen in the population of animals before slaughter.
σ_m^2. The variance of the measurement of the ultimate muscle pH in the slaughtered animal expressed in units of muscle glycogen. The parameter α is given by letting $Q = \ln(1.7)$.

Application to Measuring Repeatability Between Suppliers

Since the ultimate muscle pH in the slaughtered animal is related to meat quality it is of interest to a meat processor to be able to rank suppliers for this trait. An effective ranking system depends on a degree of repeatability within a supplier. Repeatability is defined as the intraclass correlation for the trait of interest.

The ultimate muscle pH in 1822 slaughtered animals supplied by 16 Farmer suppliers to Manawatu Beef Packers, a meat processing company in New Zealand was used to estimate the above parameters of the probability density for the ultimate muscle pH using maximum likelihood. The estimated parameters, using probability density (4) were, relating glycogen to pH up to a multiplcative constant:

$$\mu = 1.31 \pm 0.002$$
$$\sigma_x = 0.041 \pm 0.001$$
$$\sigma_m = 0.316 \pm 0.006$$

The standard errors of the parameters were obtained by a bootstrap.

Previously when the repeatability for the ultimate muscle pH within suppliers was calculated ignoring the form of the probability density the estimate was not significantly different from zero, suggesting that there was no effective ranking of suppliers. However, when the repeatability was calculated assuming a normal probability density for muscle glycogen in the animal before slaughter and estimating the parameters of this density using the above techniques the repeatability was calculated to be 0.47. An effective method of ranking suppliers can now be designed.

It is suspected that animal husbandry practices may effect the level of glycogen in the animal before slaughter, and therefore the ultimate muscle pH and the quality of the meat. However, investigations of various practices have not identified aspects which have an effect on the ultimate muscle pH in the slaughtered animal (Purchas and Keohane 1997). Applying the probability density (4) or (5) to obtain maximum likelihood estimates of the effects of interest would give more power to these investigations.

DISCUSSION

This paper has referred to a specific problem, the determination of the probability density of the observed equilibrium muscle pH in slaughtered animals. However, any system which is buffered in the manner described above and in which one component may become limiting would be expected to show similar equilibrium probability densities.

In retrospect it is clear how the technique works. Most of the variation around the peak of the density represents instrument error. That is information on differences in muscle glycogen in these animals has been lost. Information on differences between animals is to be found primarily in the tail of the frequency distribution. In effect the maximum likelihood procedure derived from equation (4) discounts the information in this part of the distribution in favour of information in the tail of the distribution.

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REFERENCES

Agha, M. and M.T. Ibrahim Maximum likelihood estimation of mixtures of distributions. Algorithm AS 203. *Applied Statistics* 32, 327-332, 1984.

Bendall, J.R.. In: *The structure and function of muscle, Vol. II* (Bourne, G.H. (Ed.)), Academic Press, New York, 243, 1973.

Tarrant, P.V. and D.E. Hood, The problems of dark-cutting in beef. Marinus Nijhoff, Den Haag, 1981.

Hosmer, D.W., A comparison of iterative maximum likelihood estimates of the parameters of a mixture of two normal distributions under three different types of sample, *Biometrics* 29, 761-770, 1973.

Purchas, R.W. and B.E. Keohane, Sources of variation in the ultimate muscle pH of M. Longissimus from prime steers. *Proceedings of the New Zealand Society of Animal Production*. 57, 271-277, 1997.