THE RELATIONSHIP BETWEEN MUSCLE GLYCOGEN STORES AND ULTIMATE pH IN COMMERCIALLY HARVESTED KANGAROOS

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Background

Following slaughter, the pH of meat declines due to the continued anaerobic catabolism of muscle glycogen to form lactic acid. Assuming that adequate levels of muscle glycogen are present at the time of slaughter, this process continues until the changing cellular environment inhibits the activity of glycolytic enzymes, usually occurring at an ultimate pH (pH_u) of around 5.5. This will occur in healthy, well-fed animals, subjected to minimal stress. In beef cattle a depletion of glycogen reserves pre-slaughter will result in limited lactic acid production post mortem, and subsequently high pH_u (Howard and Lawrie, 1956). This is associated with dark coloured beef and a reduced shelf-life of the product, which in turn leads to consumer resistance to purchasing the product (Shorthose, 1989).
Kangaroos are field harvested at night while grazing in their natural feeding habitat, with the animals being dazed with a strong spotlight before being shot. Therefore minimal stress is imposed prior to slaughter, presumably resulting in adequate glycogen stores to ensure a low and desirable pH_u.

Objectives

The objective of this study was to establish the relationship between muscle glycogen concentration at the time of slaughter and pH_u of various muscles of commercial importance from the kangaroo carcass.

Methods

Sample acquisition and laboratory analyses

Kangaroos were field harvested over 2 nights in the Hallett district of South Australia (February, 2001) by an accredited commercial kangaroo harvester. Four muscles were collected: *M. adductor* (n=28), *M. biceps femoris* (n=26), *M. vastus lateralis* (n=50), and *M. longissimus dorsi* (n=10), sourced from male and female Western Grey (*Macropus fuliginosus*) or Red kangaroos (*Macropus rufus*). The dressed weight of the carcasses ranged from 12 to 45kg, with a mean (±SD) weight of 22±9kg. Carcasses were stored at 1°C for 9 days before boning, and excised muscles were frozen at –20°C for later analysis. For glycogen determination 0.25g of frozen muscle (free of epimysium and adipose tissue) was homogenised with 30mM HCL (1:10). This homogenate was then assayed for glycogen and lactate concentrations according to the enzymatic methods of Kunst *et al* (1983) and Marbach and Weil (1967). Glycogen concentration at slaughter was represented by the sum of residual glycogen plus free glucose plus lactate concentrations determined in the post-rigor muscle. Muscle pH_u was measured using a combination electrode (TPS Brisbane, Australia).

Statistical analyses

Data were analysed using the PROC MIXED (SAS). The model for total glycogen included muscle, sex and species as fixed effects, and the interaction of muscle with sex and species. The pH_u model included the same fixed effects, plus total glycogen and its interactions with muscle and sex. Animal was included as a random effect in both models.

Results and discussion

Glycogen levels in kangaroo muscle

The mean (±SD) glycogen concentration at slaughter for muscles across species and sex were 86.1±17.73 (*M. adductor*), 88.0±18.84 (*M. biceps femoris*), 63.6±13.41 (*M. vastus lateralis*) and 90.9±22.04µmol/g (*M. longissimus dorsi)*. Shorthose (1980) and McVeigh and Tarrant (1982) reported that in healthy, well-fed domestic animals, slaughtered without undue stress, the muscle glycogen concentrations range between 60-100µmol/g wet weight. The majority of kangaroo muscles were seen to lie within this range (Figure 1).
There was a significant interaction of muscle with both species ($p<0.01$) and sex ($p<0.01$). The predicted means for each muscle within species and sex are presented in Table 1. Overall, muscles from female kangaroos contained almost 19µmol/g less glycogen than muscles from male kangaroos ($p<0.01$). The greatest effect was observed in the *M. longissimus dorsi* with over 30µmol/g less glycogen in females than in males. In this study all females had at least one suckling offspring, which may have limited substrate storage at the muscular depot due to the competing demands of pregnancy and lactation for this substrate. Alternatively, males may naturally have higher glycogen concentrations to meet the anaerobic demands of aggressive interactions between other males.
Estimated glycogen concentrations at slaughter were influenced by species, with the Western Grey exhibiting higher levels. The impact of environmental factors, such as nutrition, in which the two species were harvested may have contributed to this difference.

The pH_u of kangaroo muscle

The mean (±SD) pH_u values for muscles across species and sex were 5.78±0.09 (*M. adductor)*, 5.80±0.10 (*M. biceps femoris*), 5.88±0.12 (*M. vastus lateralis*) and 5.77±0.09 (*M. longissimus dorsi*). These values are high compared to muscle from well-fed domestic ruminant animals maintained under conditions of adequate nutrition and minimal stress prior to slaughter. Under these conditions values for pH_u for beef and sheep meats are expected to be around 5.5. Although the international standard for "dark-cutting" is around a pH_u value of 6.0, values of 5.7 in yearling cattle are considered to be dark-cutting both from an industry and consumer perspective (Shorthose, 1989). The Meat Standards Australia grading scheme has a cut off at 5.7 for beef (Ferguson *et al*., 1999). Using these standards, there is little doubt that the values measured within this study fall within the range generally considered as being "dark-cutters".

The relationship between muscle glycogen and pH_u

Tarrant (1989) reported that below a critical glycogen level (at slaughter) of 57µmol/g the pH_u of meat will be higher, resulting in reduced carcass quality characteristics such as dark coloured meat and poor keeping quality. Shorthose (1980) reported this critical threshold level as 40µmol/g. Above these glycogen concentrations, pH_u is independent of muscle glycogen concentration and generally should fall to 5.5. This study showed that kangaroo glycogen levels at slaughter were predominantly above these critical concentrations described in beef, yet the pH_u values were high, and equivalent to values of "dark-cutters" in beef (Figure 1.). The relationship between total glycogen and pHu differed between muscles and sexes ($p<0.01$). Irrespective, the results indicate that there seems to have been adequate glycogen stores at

slaughter to ensure a low pH_u in kangaroo muscle. Furthermore, residual glycogen values ranged from 6.5 – 91.8µmol/g (pooled for muscle, sex and species), with a mean (±SD) of 49.5±18.3µmol/g, indicating that muscle glycogen levels at slaughter were not exhausted during *post-mortem* glycolysis. Thus cessation of pH fall *post-mortem* occurred under conditions when glycogen was not limiting. Various factors have been suggested to influence pH_u when glycogen is not limiting. These include initial metabolite concentrations of creatine phosphate, ATP, and lactate, as well as the buffering capacity of muscle (Vetharaniam and Daly, 2000), and the availability of adenosine monophosphate (Scopes, 1971). Given the species differences, in particular the anaerobic exercise involved in the locomotion of the kangaroo compared to the less strenuous, aerobic, nature of locomotion in sheep and cattle, it is possible that buffering capacities of the muscles would differ. Figure 1 supports this notion, with the glycogen concentrations in excess of 60µmol/g demonstrating little relationship with pH_u.

Conclusions

Muscle glycogen concentrations differed significantly between species and sex within the carcass of the kangaroo. In general the glycogen concentrations were above 60µmol/g, which should have been adequate to achieve an pH_u of 5.5, as seen in sheep and cattle. However, pH_u rarely fell below 5.7, suggesting a difference in the metabolic nature of kangaroo muscle compared to sheep and cattle.

Pertinent Literature

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Table 1. Predicted means for glycogen concentrations (µmol/g wet weight ± std. err.) in different muscles from male and female Red (*Macropus rufus*) and Western Grey (*Macropus fuliginosus*) kangaroos

Muscle	Western Grey	Red	Female	Male
M. adductor	88.35 ± 3.00	81.65 ± 2.99	74.28 ± 3.13	95.72 ± 2.85
M. biceps femoris	88.91 ± 3.00	82.39 ± 3.03	78.34 ± 3.18	92.97 ± 2.85
M. longissimus dorsi	89.88 ± 3.16	74.10 ± 3.09	65.72 ± 3.38	98.26 ± 2.91
M. vastus lateralis	65.56 ± 3.06	61.27 ± 2.95	60.39 ± 3.12	66.44 ± 2.89

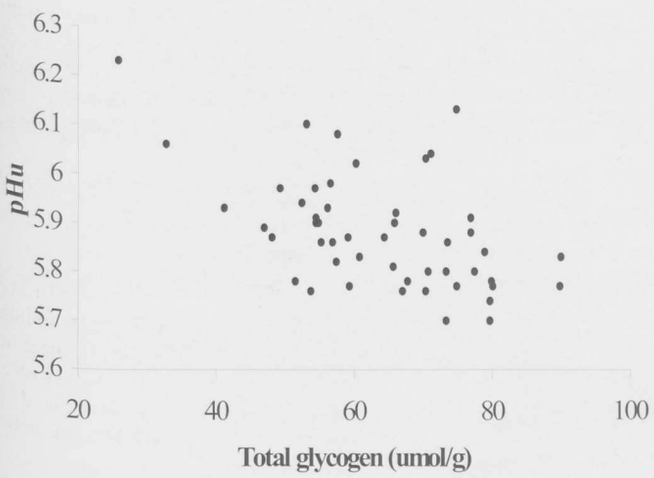

Fig 1. The relationship for total glycogen and resulting ultimate pH in the *M. vastus lateralis* of the kangaroo.