

## **The Effects of Carcass Conditioning on Shear Force Values and Water Holding Capacity of Different Skeletal Muscles of Malaysian Indigenous (MALIN) Sheep and the Changes in Their pH and Glycogen Contents**

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**Abstract:** This study investigated the effects of carcass conditioning on shear force values and water holding capacity of various major skeletal muscles (Infraspinatus, Supraspinatus, Triceps brachii, Longissimus dorsi, Rectus femoris, Vastus lateralis, Semitendinosus, Semimembranosus and Adductor femoris) obtained from a total of 18, 1 year old Malaysian Indigenous rams. It also studied the effect of conditioning on changes in their pH and glycogen contents. Sequel to the conditioning, muscle samples were analysed for shear force values and water holding capacity. The postmortem conditioning resulted in significant decline ( $p > 0.05$ ) in muscle pH, glycogen, shear force values and drip loss while the cooking loss remained unaffected. Statistically, there was no interaction ( $p > 0.05$ ) between the conditioning period and muscle type and this indicates that the effects of conditioning on muscle pH, shear force values, drip loss and cooking loss were independent of the muscle type. Meanwhile, its effect on glycogen was influenced by the muscle type.

**Key words:** Tenderness, water holding capacity, postmortem, muscle, sequel, Malaysia

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### **INTRODUCTION**

Tenderness and water holding capacity are among the most important meat quality traits in meat production, processing and preservation as a result of carcass conditioning (Melody *et al.*, 2004). The period of conditioning varies between species (14-21 days in cattle, 7-14 days in sheep and goat and 4-7 days in pigs) particularly to ensure that the ultimate tenderness is attained. Postmortem factors such as temperature, sarcomere length and myofibrillar proteolysis which affect variability in meat tenderness have been reported (Bruce and Ball, 1990). There are muscles which attain their ultimate tenderness earlier than the others throughout a period of conditioning. These behaviours have been reported (Smith *et al.*, 1978) in beef muscle after 5, 11 and 28 days of storage at 4°C, respectively. Apart from rigor development, muscle fibre characteristics have also been implicated with the variability in tenderness and other quality traits among various skeletal muscles (Maltin *et al.*, 2003).

The effects of pH and glycogen conversion to lactic acid are of high significance in post mortem changes as they are entrenched in the process of rigor mortis. The pH range of 5.6-5.8 have been reported (Hannula and

Puolanne, 2004) to aid the completion of muscles to meat conversion during the process of rigor mortis. Normally, the pH in the muscle decrease from pH 7.0 upon slaughter to approximately pH 5.3-5.8 (Hannula and Puolanne, 2004). Typically, sheep and goats usually complete their pH declines in 18-24 h post mortem (Smulders *et al.*, 1990). As the pH drops, it nears the iso-electric point. At this point, all of the negatively and positively charged amino acid side chains equal which cause the maximal attraction between the two. This attraction holds the filaments closely together and does not allow any water to get in greatly reducing the water holding capacity (Smulders *et al.*, 2006). Temperature of storage can affect this enzymatic degradation as well as other factors including pH, muscle fibre type, amount and degree of cross-linking of connective tissue and animal species (Smulders *et al.*, 2006). The conversion of glycogen to lactic acid will continue until a pH is reached when the enzymes affecting the breakdown become inactivated. In typical mammalian muscles, this pH is about 5.4-5.7 (Hannula and Puolanne, 2004). Glycogen is generally considered to be absent at pH reading above this level but certain muscle may have as much as 1% residual glycogen when the ultimate pH is above 6 (Lawrie, 1998). The effects of postmortem conditioning on meat

tenderness have been extensively studied in chicken (Chou *et al.*, 1994), porcine (Veiseth *et al.*, 2001), ovine (Hopkins and Thompson, 2002) and bovine (De Huidobro *et al.*, 2003) muscles. Nevertheless, published findings on the effects of postmortem conditioning on meat tenderness and water holding capacity particularly in tropical sheep breed are still limited. Thus, the present study was carried out to determine the effects of postmortem conditioning on tenderness and water holding capacity of nine major skeletal muscles of Malaysian Indigenous (MALIN) sheep and the changes in their pH and glycogen contents.

## MATERIALS AND METHODS

**Slaughter, muscle sampling and conditioning:** A total of 18, 1 year old Malaysian Indigenous (MALIN) rams were slaughtered at a research abattoir located at the Department of Animal Science, Universiti Putra Malaysia. The animal handling and slaughter process procedures were carried out following the guidelines of Research Policy of the Universiti Putra Malaysia on animal ethics and Malaysian Standard 1500:2004 on halal food production, preparation, handling and storage, respectively (DSM, 2004). Immediately after evisceration, the carcasses were subjected to conditioning at 4°C and subsequent samplings. The accessibility, size and economic importance are the criteria which led to the selection of Supraspinatus (SS), Infraspinatus (IS) and Triceps Brachii (TB) muscles of the forequarter and Longissimus Dorsi (LD), Adductor (A), Rectus Femoris (RF), Semimembranosus (SM), Semitendinosus (ST) and Vastus Lateralis (VL) muscles of the hindquarter in this study. Muscle chops of approximately 3 cm thick were dissected out from each specific muscle and assigned for the textural assessment while sub samples of each (approximately 30 g) were subjected to water holding capacity determination. The 0 h or unconditioned (pre rigor) steaks were immediately collected, vacuum packaged and labelled accordingly. After 24 h of carcass conditioning (1 day postmortem), the second set of muscle chops of similar thickness were also removed, trimmed off of any visible fat and connective tissue, labelled, vacuum packaged and assigned as 1 day postmortem samples. The remaining carcasses were subjected to 6 days of further conditioning before the third sub samples (7 days postmortem) were taken, prepared and stored as described earlier. All samples (0, 24 h and 7 days postmortem) were blast frozen and stored at -80°C until subsequent shear force, drip loss and cooking loss determination.

**Shear force analysis:** Meat tenderness was objectively measured by shear force analysis based on the mechanical force required to shear the muscle fibres of a cooked meat sample. The textural assessment of cooked meat tenderness was carried out using the TA.HD plus® texture analyser (Stable Micro System, Surrey, UK) fitted with a Warner Bratzler blade set. The entire samples preparation was carried out according to the procedures earlier reported (Sazili *et al.*, 2005). Muscle chops for shear force determination were thawed overnight at 4°C. Chops were cooked until an internal temperature of 78°C was reached and cooking was maintained for a further 10 min before cooling them overnight at 4°C. At least 6 cores (1.27 cm diameter) were removed from each cooked sample following as close to the longitudinal orientation of the muscle fibres as possible. Each core was placed on the base plate of the texture analyser and sheared once in the centre and perpendicular to the longitudinal direction of the fibres (Brown *et al.*, 1998). Warner-Bratzler shear force values were reported as the mean of all core values of each individual sample. A lower shear force value (kg) indicates more tender meat whereas tougher meat was indicated by a higher shear force result.

**Drip loss and cooking loss determination:** The determinations of drip loss and cooking loss were carried out according to the techniques described by Honikel (1998). Where W1 is the initial weight; W2 is the weight of raw sample and W3 is the final weight, the percentages of drip loss and cooking loss were calculated using the following equations:

$$\text{Drip loss (\%)} = [(W1 - W2) \div W1] \times 100$$

$$\text{Cooking loss (\%)} = [(W2 - W3) \div W2] \times 100$$

**Muscle pH determination:** The determination of muscle pH following to 0, 1 and 7 days of postmortem conditioning was carried out by first homogenizing for 15 sec, 0.5 g of pulverized muscle tissue in 10 mL of deionised water in the presence of 150 mM KCl and 5 mM sodium iodoacetate (Merck Schuchardt OHG, Germany). The use of sodium iodoacetate was to inhibit further glycolysis which if not stopped would have resulted in further decline of pH during the measurement. A glass electrode attached to a hand held pH meter (Mettler Toledo, USA) was used to measure the pH of the resulted homogenates (Bendall, 1975).

**Muscle glycogen analysis:** Meanwhile, the concentrations of available glycogen from the nine different muscles conditioned for 0, 1 and 7 days were enzymatically determined according to the method described by Dreiling *et al.* (1987).

**Statistical analysis:** The data generated in the present study were analysed using the General Linear Models Procedure of the Statistical Analysis System (SAS, 2000). The differences between means were analysed by Duncan's Multiple Range test. Where there is no interaction between conditioning and muscle type, the means of each individual muscle were pooled and analysed. All statements of significance were based on  $p < 0.05$ .

## RESULTS AND DISCUSSION

**Shear force values:** There were differences ( $p < 0.05$ ) in shear force values across the nine different skeletal muscles investigated in this study. In comparison with the RF, ST, TB, A, SS and LD muscles, the highest shear force values ( $p < 0.05$ ) were exhibited by the VL and SM muscles while the lowest shear force values ( $p < 0.05$ ) were observed in the RF and IS muscles (Fig. 1). In general, this is in agreement with a previous report (Sazili *et al.*, 2005) on significant differences in shear force values between LD, TFL (Tensor Fasciae Latae), ST and SS muscles in growing lambs. In the present study, there was no interaction between conditioning and muscle type and thus, the mean shear force values of all individual muscles were pooled (Fig. 2). As expected, the highest shear force values were exhibited by the unconditioned samples (0 day) which have significantly declined after 1 and 7 days of conditioning (Fig. 2). The absence of interaction ( $p > 0.05$ ) between conditioning and the muscle type indicates that the effects of conditioning on shear force values were not influenced by the muscle type.

**Drip loss and cooking loss:** The percentages of drip loss (Fig. 3) and cooking loss (Fig. 4) differed significantly ( $p < 0.05$ ) between the nine different muscles. The highest

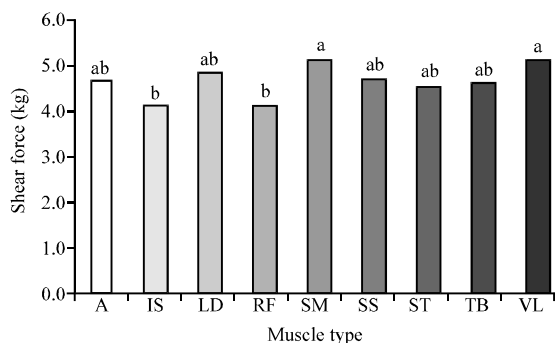


Fig. 1: Differences in shear force values of different skeletal muscles in sheep; A = Adductor, IS = Infraspinatus, LD = Longissimus Dorsi, RF = Rectus Femoris, SM = Semimembranosus, SS = Supraspinatus, ST = Semitendinosus, TB = Triceps Brachii, VL = Vastus Lateralis. Means with different letter differ significantly ( $p < 0.05$ )

and lowest percentage of drip loss was indicated by the A and IS muscle, respectively (Fig. 3). Meanwhile, compared to the A, LD, SM and TB muscles, the lowest percentage of cooking loss ( $p < 0.05$ ) was observed in the IS muscle (Fig. 4). Similar to the shear force results, the effects of conditioning on drip loss and cooking loss were also found to be independent of the muscle type and these were statistically evident by the absence of interaction between conditioning and muscle type. In general, the percentage of drip loss declined throughout the 7 days of conditioning (Fig. 5). The highest percentage was exhibited by the unconditioned samples and this could be explained by improvement in water holding capacity during the conditioning as reported earlier (Boakye and Mittal, 1993).

There are three types of water in muscle; each differing in degree of its freedom (Kolczak *et al.*, 2003). The bound water has reduced mobility and is very resistant to freezing and evaporation by heat. This water changes very little in post rigor muscles (Offer and Knight, 1988). The second type of water found in skeletal muscle is called immobilized water. These water molecules may be held either by steric effects or attraction to the

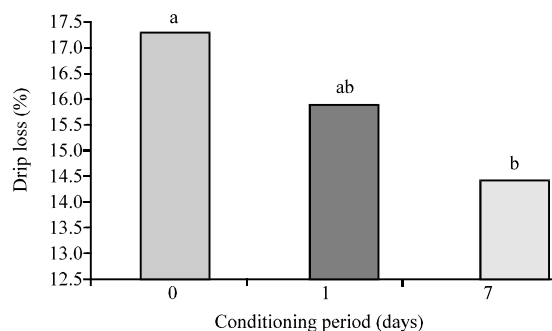


Fig. 2: Effects of postmortem conditioning on shear force values; the mean drip loss percentages of 9 different skeletal muscles were pooled since, there was no interaction between the muscle type and conditioning period. Pooled means with different letter differ significantly ( $p < 0.05$ )

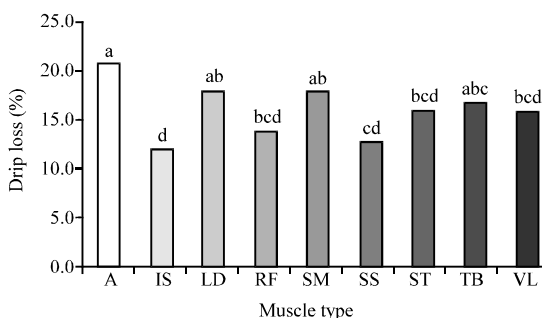


Fig. 3: Differences in drip loss of different skeletal muscles in sheep

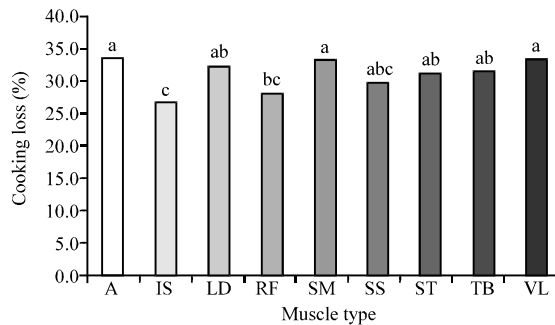


Fig. 4: Differences in cooking loss of different skeletal muscles in sheep

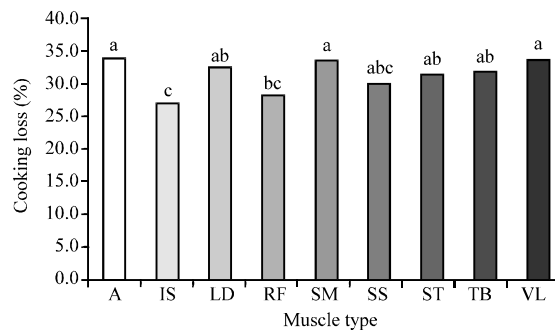


Fig. 5: Effects of postmortem conditioning on drip loss; the mean drip loss percentages of 9 different skeletal muscles were pooled since, there was no interaction between the muscle type and conditioning period. Pooled means with different letter differ significantly ( $p < 0.05$ )

bound water and held within the structure of the muscle but not bound to proteins (Huff-Lonergan and Lonergan, 2005).

The improved WHC could be due to the proteolytic degradation of cytoskeletal proteins which has subsequently caused swelling of the myofibrils and allowed the meat to retain water (Huff-Lonergan and Lonergan, 2005; Kristensen, 2001). It has been hypothesised that degradation of the cytoskeletal proteins during conditioning would increase WHC of meat by removing inter-myofibrillar and costameric connections and thereby reduce or remove the linkage between the rigor-induced lateral shrinkage of myofibrils and shrinkage of the whole muscle fibre. During post mortem conditioning, muscle structures become looser because of degradation of myofibrillar and cytoskeletal proteins (Huff-Lonergan and Lonergan, 2005; Kristensen, 2001; Koochmaraie, 1996) as well as intramuscular collagen (Koochmaraie, 1996). A previous Nuclear Magnetic Resonance study indicated that the amount of water which was tightly trapped in protein networks increases

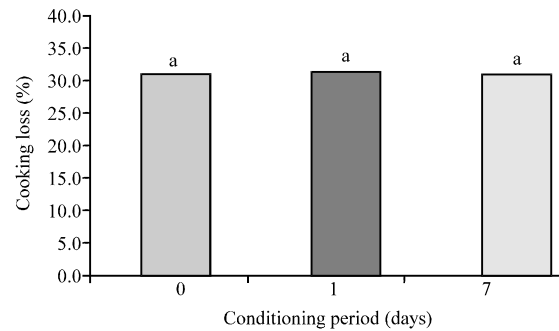


Fig. 6: Effects of postmortem conditioning on cooking loss; the mean cooking loss percentages of 9 different skeletal muscles were pooled since, there was no interaction between the muscle type and conditioning period. Pooled means with different letter differ significantly ( $p < 0.05$ )

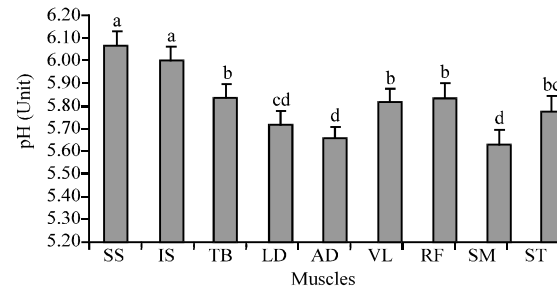


Fig. 7: Differences in pH values among different skeletal muscles in sheep

with ageing (Purslow, 2005). However, the pooled percentages of cooking loss remained unaffected by the postmortem conditioning (Fig. 6).

**Muscle pH:** There were differences in pH values (Fig. 7) among those muscles investigated. The muscles can be grouped into three significant groups in decreasing order of pH values, namely SS and IS (6.06 and 5.99), TB, VL and RF (5.83, 5.81 and 5.83) and AD and SM (5.66 and 5.63), respectively. However, the pH value of ST muscle was not significantly different compared to TB, VL, RF and LD. Besides being not significantly different compared the pH values of ST, the LD muscle was also not different ( $p > 0.05$ ) in comparison with that of AD and SM muscles. It was also observed that there was no interaction present between the conditioning period and muscle type. Hence, the mean pH values of all muscle types were pooled as muscle pH at 0, 1 and 7 days postmortem (Fig. 8). As expected, a significantly higher ( $p < 0.05$ ) pH was observed in the pre rigor (0 day) muscle samples compared to those subjected to 1 and 7 days of conditioning (post rigor muscles). In addition, the pooled

muscle pH values at 7 days postmortem have significantly dropped to lower pH values ( $p < 0.05$ ) than the 1 day conditioned samples (Fig. 8). Although, there was a significant difference in pH values between the 1 day and 7 days conditioned samples, the magnitude of drop from day 1-7 seems to be very marginal. The effects of conditioning on pH of skeletal muscles of different animals have been reported (Purslow, 2005). The pH trend observed in this study is similar to those reported for beef and sheep meat (Hopkins and Thompson, 2002).

Despite being significantly different across the various skeletal muscles, the changes in pH during postmortem conditioning were found to be independent of the muscle type. The decline in muscle pH throughout the 7 days of conditioning occurred in all muscles. This indicates that all the 9 muscles, despite showing differences in their initial glycogen concentration and pH value have completed their rigor development at the same time within 24 h post-mortem when subjected to conditioning at 4°C. Therefore, the optimum conditioning period at 4°C for MALIN sheep carcasses would be about 24 h post-mortem. The completion of rigor development

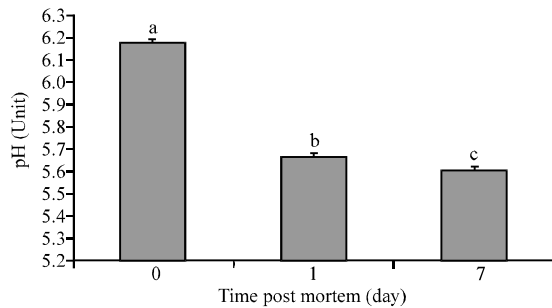


Fig. 8: Effects of conditioning on pH of different skeletal muscles in sheep; the mean pH values of 9 different skeletal muscles were pooled since, there was no interaction between the muscle type and conditioning period. Pooled means with different letter differ significantly ( $p < 0.05$ )

within the same period for different muscles with different initial pH values and glycogen concentration showed that the rate of glycolytic activity varies with different muscle. This is only possible if the metabolic and contractile properties of the muscles are different. It has been reported (Purslow, 2005) that the pH of most animal muscles decline from basic to acidic as the conditioning days increases. Therefore, the results obtained during postmortem conditioning in this study in relation to pH changes agree with the previous findings (Purslow, 2005).

**Muscle glycogen content:** The presence of interaction between conditioning and muscle type ( $p < 0.05$ ) indicates that the differences in glycogen content across the 9 different muscles were dependent on the postmortem conditioning time. In general, the glycogen content also differed significantly among the 9 different muscles. However, significant differences in glycogen content amongst the 9 muscles only occurred in the pre rigor or unconditioned (0 h) samples (Table 1). Of the unconditioned and pre rigor (0 h) samples, the highest glycogen level was observed in the IS muscle ( $19.2 \pm 4.0 \text{ mg g}^{-1}$ ) while the lowest level was exhibited by the ST muscle ( $7.24 \pm 1.58 \text{ mg g}^{-1}$ ). The magnitude of difference between the highest (IS) and the lowest (ST) glycogen level is  $11.96 \text{ mg g}^{-1}$  tissue which was calculated as approximately 63.3%. From the highest to the lowest, the initial muscle glycogen content levels can be ranked as follows: IS ( $19.20 \pm 4.0 \text{ mg g}^{-1}$ ) > VL ( $14.31 \pm 1.87 \text{ mg g}^{-1}$ ) > RF ( $13.98 \pm 0.79 \text{ mg g}^{-1}$ ) > AD ( $12.65 \pm 3.55 \text{ mg g}^{-1}$ ) > TB ( $12.23 \pm 3.18 \text{ mg g}^{-1}$ ) > LD ( $11.32 \pm 2.36 \text{ mg g}^{-1}$ ) > SM ( $9.07 \pm 3.85 \text{ mg g}^{-1}$ ) > SS ( $8.32 \pm 1.15 \text{ mg g}^{-1}$ ) > ST ( $7.24 \pm 1.58 \text{ mg g}^{-1}$ ). The magnitude of glycogen depletion in different skeletal muscles over the first 24 h postmortem were calculated and derived based on the differences in glycogen content between 0 day and 1 day (24 h) postmortem and this was done in order to estimate the depletion rate in each muscle. The fastest glycogen depletion rate within 24 h postmortem

Table 1: Effects of postmortem conditioning on muscle glycogen content of different skeletal muscles in sheep

Muscles	Post mortem conditioning (days)					
	0		1		7	
	Glycogen ( $\text{mg g}^{-1}$ tissue)	SD	Glycogen ( $\text{mg g}^{-1}$ tissue)	SD	Glycogen ( $\text{mg g}^{-1}$ tissue)	SD
AD	12.65 <sup>bcd</sup>	3.55	5.82 <sup>gh</sup>	0.43	4.99 <sup>h</sup>	0.56
IS	19.20 <sup>a</sup>	4.00	6.15 <sup>gh</sup>	0.50	5.73 <sup>gh</sup>	1.00
LD	11.32 <sup>d</sup>	2.36	5.90 <sup>gh</sup>	0.93	5.57 <sup>gh</sup>	0.69
RF	13.98 <sup>bc</sup>	0.79	6.15 <sup>gh</sup>	0.50	5.40 <sup>gh</sup>	2.14
SM	9.07 <sup>e</sup>	3.85	5.82 <sup>gh</sup>	0.36	5.73 <sup>gh</sup>	0.75
SS	8.32 <sup>ef</sup>	1.15	6.57 <sup>gh</sup>	0.50	6.15 <sup>gh</sup>	1.17
ST	7.24 <sup>efgh</sup>	1.58	5.40 <sup>gh</sup>	0.35	5.15 <sup>gh</sup>	0.90
TB	12.23 <sup>cd</sup>	3.18	7.40 <sup>fg</sup>	3.95	5.65 <sup>gh</sup>	0.97
VL	14.31 <sup>b</sup>	1.87	5.57 <sup>gh</sup>	0.69	5.07 <sup>h</sup>	0.71

Means within a row or a column with different superscripts differ significantly ( $p < 0.05$ )

occurred in the IS (13.05 mg g<sup>-1</sup> tissue) followed by VL (8.74 mg g<sup>-1</sup> tissue), RF (7.83 mg g<sup>-1</sup> tissue), AD (6.83 mg g<sup>-1</sup> tissue), LD (5.42 mg g<sup>-1</sup> tissue), TB (4.83 mg g<sup>-1</sup> tissue), SM (3.25 mg g<sup>-1</sup> tissue), ST (1.84 mg g<sup>-1</sup> tissue) and SS (1.75 mg g<sup>-1</sup> tissue). Unlike the other muscles, the rate of glycogen depletion as exhibited by IS muscle was not in agreement with (Bertram *et al.*, 2004) who classified the muscle as slow oxidative of which would undergo a slower glycogen breakdown. However, it is important to note that the previous classification was solely based on the histochemical fibre type distribution in cattle.

From Table 1, it is unlikely that the subsequent significant pH decline of muscles at day 7 of conditioning period was due to the postmortem glycolysis which occurred during the subsequent 6 days of postmortem conditioning since, there was no further drop in glycogen content ( $p > 0.05$ ) during this period. The subsequent changes in muscle pH could possibly be associated with metabolites produced during microbial activities. However, the influences of other underlying factors are yet to be researched.

In this study, the differences in reactions and responses given by those muscles to the pre slaughter handling and activities could have influenced the observed glycogen content particularly at 0 day postmortem. It has been reported that muscles involved in maintaining posture are slower than those involved in locomotion in breaking down the stored muscle glycogen (Bertram *et al.*, 2004). It is well accepted that rapid glycolysis is not essential for slow oxidative muscles. In these muscles aerobic energy production is preferred because of better blood supply, higher amounts of myoglobin, more mitochondria and more active pathway from glycogen through the Krebs cycle to CO<sub>2</sub> and H<sub>2</sub>O than in the fast glycolytic ones (Totland and Kryvi, 1991). The *in vivo* glycogen content is usually lower in slow oxidative than in the fast glycolytic muscles (Talmant *et al.*, 1986; Karlsson *et al.*, 1999; Henckel *et al.*, 1997). However, the differences in glycogen content among different muscles as shown in this study could not support the earlier findings as the highest glycogen content was indicated by the IS muscle. Similarly, SM which has been categorized as one of the fast muscles contained lower glycogen content than in the other slower muscles. Thus, the researchers believed that the 0 day results were not appropriate to represent the *in vivo* or at bleeding glycogen content. The differences observed at 0 day were actually representing 30 min postmortem data where most of the muscles particularly fast muscles had undergone a rapid postmortem biochemical changes.

Besides, IS which has been previously classified as slow muscle in this study exhibited the highest glycogen

content. This could be due to the actual sampling time which has been regarded as 0 day postmortem. The 0 day samples were actually collected within 30 min postmortem. The researchers couldn't obtain the muscle samples at point of exsanguination due to the halal slaughter requirement which does not allow any intervention after bleeding until the animal is dead. That was the major limitation of the sampling. The most appropriate or the closest to *in vivo* biochemical parameters could be obtained if muscle tissue were collected in live animal through biopsy or immediately post exsanguination. Thus, the highest glycogen in IS and other muscles actually indicated the amount of available glycogen during the rigor development and did not actually reflect the available glycogen at slaughter.

## CONCLUSION

In general, the present study has demonstrated that meat tenderness, drip loss and cooking loss differed among the different skeletal muscles. Besides improving cooked meat tenderness, the carcass conditioning applied in the present study has also improved water holding capacity as indicated by reduced drip loss percentage. Also, the effects of postmortem conditioning on muscle pH are independent of the type of muscle or vice versa. The type of muscle has affected the muscle pH only at initial stage particularly at 0 day postmortem. The results suggest that the ultimate muscle pH has been reached within the 24 h (1 day) of conditioning. The study has also demonstrated that both types of muscle and the conditioning period have affected the glycogen levels. The glycogen levels of 1 day and 7 days conditioned samples were not different. The results from these studies recommended that the optimum conditioning period for MALIN sheep carcass is 1 day since, all muscle had attained their ultimate pH and glycogen level within 24 h.

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