Muscle Fiber Characteristics and Their Relationship to Water Holding Capacity of Longissimus dorsi Muscle in Brahman and Charolais Crossbred Bulls

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ABSTRACT: Muscle fiber characteristics and their relationship to water-holding capacity of longissimus dorsi (ld) muscle were studied in Brahman (BRA) and Charolais (CHA) crossbred bulls fattened under practical farm conditions. Thirty-four BRA and 34 CHA bulls were randomly selected and slaughtered at 500, 550 and 600 kg live weight. Parameters of water-holding capacity such as drip, ageing, thawing, cooking and grilling loss were determined. Muscle fiber characteristics were conducted for muscle fiber type percentage and cross-sectional areas of slow- and fast-twitch fiber types, and correlation coefficients to water-holding capacity parameters were calculated. Results showed that CHA meat had a better water-holding capacity (less ageing, thawing and grilling loss) when compared with BRA, whereas slaughter weights had no significant effects on these parameters. Furthermore, there were no significant differences between genotypes and slaughter weights in muscle fiber type percentage and cross-sectional areas of ld muscle. Slow- and fast-twitch fiber types of all experimental groups averaged 24.4 and 75.6%, respectively. Cross-sectional areas of fast-twitch fibers had almost twice the size of slow-twitch fibers (6,721 and 3,713 μm², respectively). The correlation between muscle fiber area and water-holding capacity indicated that muscles with larger fiber areas had a lower drip and ageing loss but a higher cooking and grilling loss. (Key Words: Cattle, Brahman, Charolais, Muscle Fiber Characteristics, Water Holding Capacity)

INTRODUCTION

Skeletal muscle fibers are classified, depending on their contraction rate and metabolic reaction, into slow-twitch oxidative (SO) and fast-twitch fibers, which include fast-oxidative glycolytic (FOG) and fast-twitch glycolytic (FG) fibers (Brooke and Kaiser, 1970; Peter et al., 1972). According to Tortora (2005), SO fibers have a small fiber diameter, and a high mitochondrial and myoglobin content. They contain many triglycerides and enzymes involved in the oxidative pathway. Fast-twitch fibers on the other hand, have a large fiber diameter and a low mitochondrial and myoglobin content. Between the two extremes, SO and FG fibers, FOG fibers have a moderate structural and energetical property.

The relationship between muscle fiber characteristics, size, types with post mortal biochemical processes and meat quality is well documented (Klont et al., 1998; Ryu et al., 2004; Ryu and Kim, 2005; Berri et al., 2007). However, muscle fiber characteristics are influenced by many parameters, e.g. genotype, sex, age, feed and management (Ozawa et al., 2000; Vestergaard et al., 2000; Kirchofer et al., 2002). Postnatal the apparent number of muscle fibers of each muscle does not change, while a transformation of type IIA into IIB fibers during the first few months after birth and an enlargement of muscle fiber size up to 10-fold during the first two years of age can be observed (Wegner et al., 2000). Different muscle fiber characteristics of different cattle genotypes/breeds or slaughter weights (ages) require different postmortem treatments to achieve the best ultimate meat quality. Information about muscle fiber characteristics of beef cattle in Thailand especially for native cattle upgraded by specific beef breeds is limited. Therefore, the objectives of this study were, firstly, to investigate effects of genotype and slaughter weight on histochemical muscle fiber characteristics and water holding capacity of the longissimus dorsi (ld) muscle in Brahman and Charolais crossbred bulls, and secondly, to estimate the relationship between muscle fiber characteristics and water holding capacity of the ld muscle.
MATERIALS AND METHODS

Animals and feeding
The study was conducted at a commercial fattening beef company in Chiang Mai, Northern Thailand. The experiment included 34 Brahman×Thai native (BRA) and 34 Charolais×Thai native (CHA) crossbred bulls with a mean initial weight of 319 kg (SD = 30 kg). Characteristics of Brahman×Thai native crossbred cattle are similar to purebred Brahman with a large hump over the top of the shoulder. Their ears are generally large and pendulous, and the throatlatch and dewlap have a large amount of excess skin. The colour of BRA is usually light to medium grey. Mature bulls mostly have dark areas on the neck, shoulders and lower thighs. Charolais×Thai native crossbred cattle look similar to purebred Charolais. The colour is creamy white or light brown. The hair coat is shorter than purebred and they show a small hump over the top of the shoulder. The animals were fattened under practical farm conditions and kept in stanchion barns. They were fed ad libitum on seasonal grass, rice straw, corn, corn residue (husk and cob) from the agro-industry and fresh water, and received 1 kg of concentrate per 100 kg live weight per day with a protein concentration of 12%. Mean age at start of fattening of both genotypes were 19 month and mean live weight at start of fattening was 323 kg (SD = 29.7 kg) and 316 kg (SD = 35.1 kg) for BRA and CHA, respectively. The fattening period ranged between 7 to 13 month. The animals of both genotypes were randomly selected and slaughtered at a mean live weight of 500, 550, and 600 kg, respectively. The experimental design was 2 (genotype)×3 (slaughter weight) factorial, resulting in 6 groups with 11 or 12 animals per group.

Slaughtering and water holding capacity determination
Each animal was weighed at the beginning and the end of the fattening period. When the animals reached the target slaughter weight, they were transported to a commercial slaughter house in Chiang Mai, where they were fasted for 12 h and weighed afterwards. The animals were slaughtered and dressed according to the commercial procedure. Within 1 h postmortem (pm), a sample of ld muscle between the 12th and 13th rib was taken by a 1.5 cm diameter biopsy cannula. The muscle sample was cut into cubes of 1 cm^3 and frozen in liquid nitrogen. Until subsequent histochemical analysis of the muscle fibers the samples were stored at -65°C in a freezer.

Carcasses were chilled for 24 h at 4°C. A cut of ld muscle was removed from the right carcass side between the 10th and 12th rib for subsequent evaluation. The ld cut was trimmed to remove residual adipose tissue and epimysium. A steak, 2.5 cm thick, was removed from the ld cut and used to determine 48 h drip loss at 0-4°C by the bag method according to Honikel and Hamm (1999). The remaining ld cut was weighed, packed in plastic bags and aged at 4°C for 14 days. Ageing loss was determined 7 and 14 days p.m. After 14 days of ageing, 2 more steaks with the same thickness were prepared from the ld cut and stored at -20°C until boiling and grilling loss analysis. Both steaks were thawed at 4°C for 24 h and thawing loss was calculated. One of the steaks was vacuum packed in a polyethylene bag and boiled in a water bath (WB 29, Memmert, Schwabach, Germany) at 82°C for 45 min to calculate boiling loss percentage. The other steak was grilled in an electric air-convection oven unit (MX-4011, Maxwell, Taipei, Taiwan) until the internal temperature reached 70°C to calculate grilling loss percentage.

Muscle fiber characteristics
The muscle samples from biopsy were sectioned for 12 micron thickness slice by a cryostat microtome (Leica model CM 1900, Germany) with a chamber temperature of -22°C and a knife temperature of -18°C. Two main muscle fiber types, slow-twitch and fast-twitch fiber, were distinguished by myofibrillar ATPase (acid pre-incubation) reaction according to Horak (1983). The fast-twitch oxidative glycolytic and the fast-twitch glycolytic fibers were combined. Cross-sectional areas were measured at 3 random frames. Muscle fiber numbers were counted from 3 complete bundles under the microscope (Nikon Eclipse model E600, Japan) using the “Lucia G” image analytic software (Figure 1).

Statistical analysis
Data were statistically analysed by using the general linear model procedure of the Statistical Analysis System Institute, version 9.1 (SAS Institute Inc., 2003). The data were analysed as a 2×3 factorial design with terms for genotype, slaughter weight, interaction between genotype and slaughter weight, and error. Multiple comparisons were done with the Tukey test with a significance level of p<0.05. Pooled standard error (PSE) was calculated according to the formula given by Pesti (1997). Pearson’s correlation coefficients for water holding capacity characteristics and muscle fiber traits were analysed using the CORR procedure.

RESULTS

Water holding capacity parameters
Concerning water holding capacity parameters, 7-day ageing, thawing and grilling loss percentages were lower for CHA, whereas drip, 14-day ageing and boiling loss percentages were not significantly affected by genotype.
(Table 1). Slaughter weight had no significant effect on water holding capacity.

**Muscle fiber characteristics**

Both genotype and slaughter weight had no significant effect on muscle fiber type percentage (Table 2). Percentages of slow- and fast-twitch fibers averaged 24.4 and 75.6% over all experimental groups, respectively. Muscle fiber cross-sectional area was neither affected by genotype nor by slaughter weight. Cross-sectional area of fast-twitch fibers was almost twice the size of slow-twitch fibers (6,721 and 3,713 μm², respectively).

**Relationship between muscle fiber characteristics and water holding capacity**

Correlation coefficients (r) between muscle fiber characteristics and water holding capacity of ld muscle of BRA and CHA are shown in Table 3. Muscle fiber type percentage had a significant relation to grilling loss. The percentage of slow-twitch fibers was positively correlated

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**Table 1.** Effects of genotype and slaughter weight on water holding capacity of longissimus dorsi muscle of Brahman (BRA) and Charolais (CHA) crossbred bulls

<table>
<thead>
<tr>
<th>Water holding capacity</th>
<th>Genotype (G)</th>
<th>PSE</th>
<th>Slaughter weight (W)</th>
<th>PSE</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRA</td>
<td>CHA</td>
<td>500 kg</td>
<td>550 kg</td>
<td>600 kg</td>
</tr>
<tr>
<td>Drip loss (%)</td>
<td>3.09</td>
<td>3.10</td>
<td>0.245</td>
<td>3.06</td>
<td>3.03</td>
</tr>
<tr>
<td>7-day ageing loss (%)</td>
<td>2.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.170</td>
<td>2.85</td>
<td>2.69</td>
</tr>
<tr>
<td>14-day ageing loss (%)</td>
<td>5.51</td>
<td>5.51</td>
<td>0.335</td>
<td>5.19</td>
<td>5.58</td>
</tr>
<tr>
<td>Thawing loss (%)</td>
<td>10.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.478</td>
<td>9.29</td>
<td>8.70</td>
</tr>
<tr>
<td>Boiling loss (%)</td>
<td>38.3</td>
<td>37.9</td>
<td>0.37</td>
<td>38.0</td>
<td>38.3</td>
</tr>
<tr>
<td>Grilling loss (%)</td>
<td>33.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.54</td>
<td>31.7</td>
<td>31.9</td>
</tr>
</tbody>
</table>

Values with different superscripts differ significantly between genotypes (*<sup>a</sup>*, **<sup>b</sup>**).

* p<0.05; ** p<0.01; ns = No significant; PSE = Pooled standard error.

**Table 2.** Effects of genotype and slaughter weight on muscle fiber characteristics of longissimus dorsi muscle of Brahman (BRA) and Charolais (CHA) crossbred bulls

<table>
<thead>
<tr>
<th>Muscle fiber characteristics</th>
<th>Genotype (G)</th>
<th>PSE</th>
<th>Slaughter weight (W)</th>
<th>PSE</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRA</td>
<td>CHA</td>
<td>500 kg</td>
<td>550 kg</td>
<td>600 kg</td>
</tr>
<tr>
<td>Fiber type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slow-twitch fiber (%)</td>
<td>25.8</td>
<td>23.0</td>
<td>0.90</td>
<td>23.7</td>
<td>24.5</td>
</tr>
<tr>
<td>Fast-twitch fiber (%)</td>
<td>74.2</td>
<td>77.0</td>
<td>0.90</td>
<td>76.3</td>
<td>75.5</td>
</tr>
<tr>
<td>Fiber cross-sectional area</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slow-twitch fiber (μm²)</td>
<td>3,608</td>
<td>3,818</td>
<td>193.6</td>
<td>3,819</td>
<td>3,775</td>
</tr>
<tr>
<td>Fast–twitch fiber (μm²)</td>
<td>6,773</td>
<td>6,670</td>
<td>242.5</td>
<td>6,689</td>
<td>6,947</td>
</tr>
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</table>

ns = No significant; PSE = Pooled standard error.
with grilling loss, whereas an inverse relation between percentage of fast-twitch fibers was observed. This indicates that muscles exhibiting a high grilling loss, have a high proportion of slow-twitch fibers and a low proportion of fast-twitch fibers. No relationship between muscle fiber type percentage and other water holding capacity parameters was observed.

Cross-sectional areas of both slow- and fast twitch fibers were negatively correlated to drip loss and 7-day ageing loss. With increasing cross-sectional areas of muscle fibers, drip and 7-day ageing loss decreased. In contrast, positive correlations were found between cross-sectional areas of both fiber types and cooking loss, and between cross-sectional area of fast-twitch fibers and grilling loss. This means that a large size of the muscle fibers is associated with a high cooking and grilling loss. No relationship between muscle fiber cross-sectional area and 14-day ageing and thawing loss were found.

**DISCUSSION**

With increasing slaughter weight, feeding time becomes longer and the animals are getting older. Therefore, an increasing slaughter weight comes along with extended feeding times and increased animal ages (Sami et al., 2004; Moreno et al., 2008).

**Water holding capacity parameters**

The percentages of 7-day ageing, thawing and grilling loss reflect that CHA had a better water holding capacity than BRA. Compared to results of Jaturasitha et al. (2009) and French et al. (2001), the boiling loss percentage in our study was higher. This may be due to the different preparation method using higher temperatures in this study. Gerhardy (1995) described that cooking loss percentage of ld steaks boiled at 75°C to an internal temperature of 75°C was higher than those boiled at 55°C to an internal temperature of 55°C and roasted at 200°C to an internal temperature of 72°C. The higher water holding capacity of CHA meat can be explained by the relatively high pH value 1 and 24 h pm of CHA meat (data not shown). Postmortem pH value was highly correlated with water holding capacity of meat (Lee et al., 2005). Rusman et al. (2003) described differences of cooking loss between breeds. Hoving-Bolink et al. (1999) found similar percentages of 7-day drip and boiling loss of longissimus muscle of Piemontese and Limousin crossbred bulls. Several studies showed an effect of slaughter weight, slaughter age or feeding time on cooking or drip loss percentage (Camfield et al., 1997; Keane and Allen, 1998).

**Muscle fiber characteristics**

To compare the present results to previous studies, slow-twitch fibers are assumed as slow-twitch oxidative (SO), type I, β red or red fibers, respectively, and fast-twitch fibers as fast-twitch oxidative glycolytic (FOG) and fast-twitch glycolytic (FG) (Peter et al., 1972), type IIA and type IIB (Brooke and Kaiser, 1970), α red and α white (Ashmore and Doerr, 1971) or intermediate and white fibers (Gauthier and Padykula, 1966).

The percentages of slow- and fast-twitch fibers of ld muscle of BRA (25.8 and 74.2%, respectively) and CHA (23.0 and 77.0%, respectively) are comparable to results of Johnston et al. (1975) and Maltin et al. (2001) who found ld muscles of Charolais cattle having percentages of slow-twitch fibers of 23 to 25% and of fast-twitch fibers of 75 to 77%. However, variations of slow- (21 to 29%) and fast-twitch fibers (71 to 79%) of ld muscle of other genotypes/breeds (Salers, Japanese Black and Holstein) have been reported (Listrat et al., 1999; Ozawa et al., 2000; Maltin et al., 2001). Similar findings of no significant effects of genotype on percentages of slow- and fast-twitch fibers in ld muscle have been reported by Johnston et al. (1975) and Maltin et al. (2001), respectively. However, an interaction between breed and feed on muscle fiber type percentage has been reported by Maltin et al. (1998) who found that Charolais and Angus bulls fed silage-based had a similar muscle fiber type percentages while bulls fed barley-based had similar percentages of SO and FOG fibers with the exception of FG fibers, which were higher in Charolais bulls. The insignificant effect of slaughter weight on muscle fiber type percentage is consistent with results of

<table>
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<tr>
<th>Table 3. Correlation coefficient (r) between muscle fiber characteristics and water holding capacity of <em>Longissimus dorsi</em> muscle of Brahman (BRA) and Charolais (CHA) crossbred bulls</th>
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<tbody>
<tr>
<td>Fiber type</td>
</tr>
<tr>
<td>Slow-twitch fiber (%)</td>
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<tr>
<td>Fast-twitch fiber (%)</td>
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<tr>
<td>Slow-twitch fiber (μm²)</td>
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<tr>
<td>Fast-twitch fiber (μm²)</td>
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</table>

Level of significant: p<0.05; ns = No significant.
Kirchofer et al. (2002) who found unchanged muscle fiber type percentage when carcass weight increased. Constant muscle fiber type percentages of cattle with increasing slaughter age (Maltin et al., 1998) or extended feeding period (Johnston et al., 1975) have been reported previously. Wegner et al. (2000) noted that after 12 month of age, muscle fiber type percentage of cattle was constant. Furthermore, Zerouala and Stickland (1991) reported that cattle at risk for dark cutting beef have a higher proportion of oxidative muscle fibers and Klont et al. (1998) stated that muscles from halothane pigs have an increased number of glycogen deplete type IIA and IIB fibers at slaughter.

Muscle fiber cross-sectional areas of ld muscle in the present study are greater than in previous studies (Brandstetter et al., 1998; Listrat et al., 1999; Ozawa et al., 2000; Kirchofer et al., 2002). This may be due to the fact that animals were older in the present study. However, when compared with semitendinosus muscle of Charolais and German Holstein bulls (Bellmann et al., 2004) and of German Angus, Galloway, Holstein Friesian and Belgian Blue bulls (Albrecht et al., 2006), the values are similar. Results of Jaturasitha et al. (2009) indicate that upgrading Thai native cattle by either Brahman or Charolais results in larger muscle fiber areas. Insignificant effects of genotype on muscle fiber cross-sectional areas have also been found previously. Maltin et al. (2001) found insignificant differences in areas of SO, FOG and FG types between Charolais and Holstein steers. Wegner et al. (2000) found no differences of muscle fiber cross-sectional areas between Galloway and Belgian Blue and between Holstein Friesian and Belgian Blue at 2 years of age. Johnston et al. (1975) found that Charolais and Angus steers had similar β red and α red fiber sizes, whereas α white fiber size was larger for Charolais. Maltin et al. (1998) found an interaction between breed and feed on muscle fiber areas. Charolais and Angus bulls fed barley-based had similar areas of SO, FOG and FG types while bulls fed silage-based had similar areas of FOG and FG type fibers except SO type fibers which were larger in Angus bulls. The insignificant effect of slaughter weight on muscle fiber cross-sectional areas is in agreement with results of Ozawa et al. (2000) and Kirchofer, et al. (2002) who found that diameters or areas of each fiber type were not associated with carcass weight. Johnston et al. (1975) found similar muscle fiber areas for all fiber types when extending the finishing period from 153 to 233 days. The unchanged muscle fiber cross-sectional areas with increasing slaughter weight may be attributed to the small differences among slaughter weights causing small differences in feeding period (±36 day) and carcass weight (±59 kg) between the extreme slaughter weights compared to previous studies which had age or feeding period differences of 120 to 180 days (Seideman et al., 1986; Maltin et al., 1998; Wegner et al., 2000). Commonly found in previous studies are increasing muscle fiber areas of all muscle fiber types with increasing slaughter age.

### Relationship between muscle fiber characteristics and water holding capacity

Muscle fiber type percentage had no significant relationship with drip, 7-day and 14-day ageing, thawing and boiling loss. Ozawa et al. (2000) found a positive relationship between percentage of α red type fiber and cooking loss in longissimus thoracis muscle of 27 month old Japanese Black steers while percentages of β red and α white type fibers showed no relationship with drip loss. Slow-twitch fiber type percentage was correlated positively with grilling loss, whereas an inverse relation between fast-twitch fiber type percentage and grilling loss was observed. Gotoh (2003) stated that type I fibers had a significant positive correlation, while type IIB fibers had a significant negative correlation with intramuscular fat. Additionally to water, fat was lost during grilling. Therefore, the positive correlation between slow-twitch fiber percentage and grilling loss could be partly explained by a higher fat content of meat with a high proportion of slow-twitch fiber. Furthermore, a relationship between muscle fiber type percentage and eating quality has been reported in which increasing numbers of SO fibers were associated with increased tenderness and juiciness, whereas increasing numbers of FG fibers were associated with increased toughness (Maltin et al., 1998).

Cross-sectional area of both slow- and fast-twitch fiber types were negatively related to drip and 7-day ageing loss and positively to boiling and grilling loss. These results reflect better water holding capacity of large fiber sizes in storage or under cool conditions but not under heat or cooking conditions. Similar findings have been reported previously. Berri et al. (2007) reported negative correlations between muscle fiber cross-sectional area and drip loss in breast muscle of broiler. The authors stated that, as the fiber size increased, the post mortem glycolytic activity of muscle decreased. As a consequence, breast muscles with large fibers exhibited the highest pH. Therefore, breast muscles with the largest fibers exhibited a darker lightness value and lower drip loss. Contrary pictures of the relationship between muscle fiber size and water holding capacity have been reported. Ozawa et al. (2000) found no significant correlation between muscle fiber area and cooking loss in longissimus thoracis muscle of 27 month old Japanese Black steers. In addition, correlations between muscle fiber area and other meat quality traits have been reported previously, whereas generally muscle fiber area was positively correlated with shear force value, lean percentage, loin eye area, lean tissue growth rate and
sensory juiciness ratings and negatively with sensory tenderness ratings, fat thickness and yellowness (b*) (Crouse et al., 1991; Čandek-Potokar et al., 1999).

In conclusion, CHA meat had better water holding capacity (less ageing, thawing and grilling loss) than BRA meat. No significant effects of slaughter weight on water holding capacity parameters and of genotype as well as slaughter weight on muscle fiber characteristics of ld muscle were recorded. The percentages of slow- and fast-twitch fiber types of all experimental groups averaged 24.4 and 75.6%, respectively. Cross-sectional areas of fast-twitch fibers had almost twice the size of slow-twitch fibers (3,713 and 6,721 μm², respectively). The correlation between cross-sectional area and water holding capacity indicated that muscles with large fiber areas had low drip and ageing loss but high cooking and grilling loss.

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