Mapping QTL for fatty acid composition that segregates between the Japanese Black and Limousin cattle breeds

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Abstract
The objective of this study was to search for quantitative trait loci (QTL) that segregate between Japanese Black and Limousin cattle breeds and affect relative amounts of saturated (SFA), mono-unsaturated (MUFA) and poly-unsaturated (PUFA) fatty acids. Six F₁ Japanese Black-Limousin cross bulls were joined with 121 F₁ females over a three-year period to produce 328 F₂ progeny. Calves, aged from 450 to 641 d (average 561 d), were harvested using standard industry procedures. After aging, 2.54 cm thick steaks were cut from the posterior end of the wholesale rib, frozen at –20 °C, and held for determination of fatty acid composition. Percentages of the individual fatty acids were classified into percentages of SFA, MUFA and PUFA. Two hundred seventeen microsatellite markers covering the 29 bovine autosomes were assayed and QTL were identified by least squares regression. Genome-wise significant QTL with additive effects on SFA (-0.61 ± 0.19%), MUFA (0.93 ± 0.19%) and PUFA (-0.52 ± 0.11%) were observed near the centromere of BTA2. Also observed were five QTL indicative of dominance effects on: MUFA (BTA9, 119 cM, -1.87 ± 0.72%; BTA22, 47 cM, 1.85 ± 0.60%) and PUFA (BTA9, 54 cM, -1.49 ± 0.42%; BTA10, 38 cM, 1.20 ± 0.35%; and BTA15, 14 cM, 1.11 ± 0.36%). Based on these results, we conclude that it may be possible to improve the healthfulness of beef by manipulating fatty acid composition using genetic markers and appropriate crossbreeding systems.

Keywords: Beef, fatty acids, Japanese Black, Limousin, quantitative trait loci, Wagyu

Meat contains a mixture of saturated (SFA), mono-unsaturated (MUFA) and poly-unsaturated (PUFA) fatty acids. Fatty acid composition in beef has received considerable interest in view of its implications for human health and meat quality characteristics (Wood et al., 2004). While unsaturated fats are beneficial when consumed in moderation, high levels of saturated fat are associated with increased serum low-density lipoprotein cholesterol concentrations and pose a risk factor for coronary heart disease (Department of Health, 1994; Katan et al., 1994, 2000). In addition to the human health implications of fatty acid composition, beef with the most desirable flavour has a lower percentage of SFA and PUFA and a higher percentage of MUFA in the muscle fat (Melton et al., 1982).

Based on experimental comparisons of Limousin and Wagyu germplasm it was concluded that these breeds differ markedly with respect to fat deposition (Mir et al., 2002, Pitchford et al., 2002). Beef from the Wagyu breed, Japanese Black, is known for its extensive intramuscular fat or marbling. In contrast, beef from Limousin is lean with little marbling. Further, Japanese Black beef has a greater absolute level of MUFA and a greater ratio of MUFA relative to SFA than other breeds (e.g. Sturdivant et al., 1992; May et al., 1993; Mir et al., 2000). These differences provide an opportunity to identify quantitative trait loci (QTL) that affect carcass quality and fatty acid composition, to enhance meat palatability and minimize human health implications. Thus, the objective of this study was to search for QTL segregating in a population derived from a cross between Japanese Black and Limousin and affecting relative amounts of SFA, MUFA
and PUFA. Further details of this project and QTL affecting carcass traits, meat quality, and absolute amounts of various fatty acids were presented previously by Alexander et al. (2007a; b).

Six F\textsubscript{1} Japanese Black-Limousin cross bulls were joined with 121 F\textsubscript{1} females over a three-year period to produce 328 F\textsubscript{2} progeny. Calves were reared by their dams until weaning at approximately 175 d of age (SD = 14 d). Each year, before harvest and within sex, calves were randomly assigned to a harvest date in groups of 8 to 11 hd per d. After weaning, the calves were managed in a two-phase system: a growing phase with diet composition of 50 to 54\% DM, 14.4 to 15.6\% CP, and 4.44 to 4.94 MJ/kg of NE\textsubscript{g}, and a finishing phase with diet composition of 68 to 70\% DM, 11.6 to 13.4\% CP, and 5.27 to 5.48 MJ/kg of NE\textsubscript{g}. The finishing diet was fed for a minimum of 113 d until the calves were harvested. Within year and sex, groups of calves were harvested at two to three week intervals. Thus, the final group harvested each year had been consuming the finishing diet for at least 210 d. Calves, aged from 450 to 641 d (average 561 d), were transported to the abattoir the afternoon before harvest, held overnight with water and without feed, and harvested the next morning using standard industry procedures. To insure the ethical treatment of animals, all protocols involving the use of animals were approved by the Fort Keogh Livestock and Range Research Laboratory Animal Care and Use Committee. Two days post mortem, the wholesale rib was removed, vacuum packaged, and aged for 14 d at 2 °C. After aging, a three-rib section and four 2.54 cm thick steaks were cut from the posterior end of the wholesale rib, then individually vacuum packaged, frozen at –20 °C, and held for further analyses.

Two steaks were transported to University of Wyoming for determination of fatty acid composition as described by Rule et al. (2002). Briefly, the entire core of the Longissimus dorsi was sampled (i.e., devoid of trim fat and extraneous muscles) by dicing the muscle into 1.0 cm cubes while the muscle was semi-frozen, and weighed into pre-weighted, plastic cups with perforated lids. All samples were freeze-dried (Genesis 25 freeze dryer, The VirTis Co., Gardiner, NY), and then ground and homogenized using a home-style electric coffee grinder. Samples were packed into 20 mL plastic vials and sealed to inhibit exposure to air, and then stored at –80 °C until analyzed for fatty acids, which occurred within 2 to 4 wks of freeze drying. Approximately 150 mg of dried muscle were weighed, in duplicate, into 16 mm × 125 mm screw-capped tubes that contained 1.0 mg of tridecanoic acid as internal standard, and then subjected to direct saponification as described by Rule et al. (2002). Samples were reacted with 4.0 mL of 1.18 M KOH in ethanol at 90 °C along with frequent vortex-mixing (two to three times per minute for 3 sec each time) until the sample was completely dissolved, except for insoluble collagen that appeared as a white powder in suspension upon mixing. After about 45 min, tubes were cooled, 2.0 mL water were added and nonsaponified lipids extracted with 2.0 mL of hexane and removed. One millilitre of concentrated HCl was added to the original tubes and fatty acids extracted in 2.0 mL of hexane for fatty acid methyl ester (FAME) preparation, which was carried out according to Rule et al. (2002) using methanolic HCl as a catalyst. Analysis of conjugated linoleic acid (CLA) is hampered by use of acid catalysts because of partial geometric isomerization of cis-9, trans-11 CLA to trans-9, trans-11 CLA (Yamasake et al., 1999) and degradation of CLA to aliphatic methoxy artifacts (Kramer et al., 1997). However, Murrieta et al. (2003) demonstrated that dietary treatment effects on CLA in ovine muscle were maintained when acid catalysts were used for FAME preparation, despite up to 20\% loss of cis-9, trans-11 CLA. Preparation of FAME from nonesterified free fatty acids (NEFA) requires the use of the acid catalyst because alkaline catalysts do not react with NEFA to form fatty acid methyl esters (Christie, 1982). For the current study, freeze dried muscle samples were chosen at random from approximately 5\% of the samples for FAME preparation using methanolic KOH, which does not affect CLA proportions. We did not observe losses of CLA in the samples analyzed (data not shown). Generally, we have observed either minimal or no loss of CLA in samples containing low concentrations (about 0.5 mg per 100 mg of total fatty acids) of this fatty acid. Quantities of the various fatty acids were summarized by calculating relative amounts of SFA, MUFA and PUFA.

DNA was extracted from semen of sires of the F\textsubscript{1} bulls and from white blood cells of the F\textsubscript{1} bulls, F\textsubscript{1} females, and F\textsubscript{2} calves using standard protocols (e.g. Ausubel et al., 1994). Touchdown PCR was performed in MJ Research (Waltham, MA, USA) thermocyclers as described in http://biolibrary.licor.com/htdocs/RnP/LabPrim.jsp. All genotypes were collected on a LiCor 4200 DNA Analysis System (Lincoln, NE, USA). Genotypes were independently scored by two individuals. PCR was repeated for discrepancies that could not be resolved. Anomalous genotypes were detected using GenoProb (Thallman et al., 2001a,b). Genetic linkage maps were constructed using CRI-MAP (Green et al., 1990; http://compgen.rutgers.edu/multimap/crimap/). Two hundred seventeen markers covering the 29 bovine
autosomes were chosen from http://www.marc.usda.gov/genome/cattle/cattle.html based on marker position, suitability for multiplex reactions, ease of scoring and number of alleles. Distance between markers was less in regions containing previously detected QTL for the amounts of various fatty acids.

Quantitative trait loci were identified by least squares regression using the F	extsuperscript{2} option of the QTL Express software for analysis of quantitative trait data from outbred populations (Seaton et al., 2002). An F-statistic profile was generated at 1-cM intervals for each chromosome. Effects of one additive QTL and one QTL with additive and dominance effects were modelled in separate analyses. The phenotypes were simultaneously adjusted for fixed effects of year and sex, and covariate age at harvest. Thus, given the random assignment of animals to harvest dates and the statistical model used to analyze the data, inference here is to an age-constant endpoint. Observed significance levels were adjusted to a genome-wide basis following Cheverud (2001). Ninety-five percent confidence intervals (CI) for the QTL map locations were calculated as described by Darvasi & Soller (1997). The hypothesis of two QTL versus one QTL was also tested for each phenotype – chromosome combination, again using QTL Express.

Genome-wise significant QTL with additive effects on SFA (-0.61 ± 0.19%, P = 0.04) at 4 cM (CI = 54 cM), MUFA (0.93 ± 0.19%, P < 0.01) also at 4 cM (CI = 24 cM), and PUFA (-0.52 ± 0.11%, P < 0.01) at 11 cM (CI = 28 cM) were observed situated between markers DIK4469 and ILISTS026 on BTA2 (Figure 1). Substitution of an allele from Japanese Black for one from Limousin decreased percentages of SFA and PUFA and increased MUFA content. This QTL was also identified by Alexander et al., (2007b) as affecting fatty acid content, marbling, and flavour. Previous studies have positively associated desirable flavour with the MUFA oleate (Dryden & Marchello, 1970; Westerling & Hendrick, 1979; Melton et al., 1982).

Figure 1 BTA2 F-statistic profiles for relative amounts of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), and poly-unsaturated fatty acids (PUFA). From centromere to telomere, markers were TGLA44, DIK4469, ILISTS026, TGLA431, DIK1172, CSFM050, TEXAN-2, TGLA377, RM356, ILISTS050, BMS1866, BMS2267 and BM2113 (shown as triangles). The genome-wide significance threshold is F = 9.46.

Growth differentiation factor 8, or myostatin is co-located with the observed QTL on BTA2 and has profound effects on muscularity and beef composition (McPherron et al., 1997; Smith et al., 1997). Raes et al. (2001) suggested the polyunsaturated/saturated fatty acid ratio increased with low intramuscular fat content, likely because of the decreased saturated fatty acids associated with decreased intramuscular adipose tissue. Several mutations in myostatin cause exceptional muscle development or muscle hypertrophy which is commonly referred to as double muscling (McPherron & Lee, 1997). However, neither Limousin nor Japanese Black are thought to be double muscled and the classical phenotype was not observed in the F	extsuperscript{2} generation. Sequencing the myostatin gene revealed five of the F	extsubscript{1} sires were heterozygous for the previously
reported (Sellick et al., 2007) transversion (g.433C>A) resulting in an amino acid change of phenylalanine to leucine at position 94 of the myostatin protein.

Two QTL indicative of dominance effects were observed on BTA9. A QTL located at 54 cM (CI = 39 cM) affected PUFA (-1.49 ± 0.42%, P < 0.01). The second QTL affected MUFA (-1.87 ± 0.72%, P = 0.09) and was located at 119 cM (CI = 67 cM). As the total of SFA, MUFA, and PUFA is 100%, these results were potentially indicative of two QTL on BTA9. However, the two QTL model was only nominally better than the single QTL model for MUFA (P = 0.04). In each case, substitution of an allele from Japanese Black for one from Limousin indicated a decrease in the proportion of the respective group of fatty acids. Three additional QTL that were also suggestive of dominance effects on the relative amounts of fatty acids were also detected. QTL affecting PUFA content were observed at 38 cM (CI = 62 cM) on BTA10 (1.20 ± 0.35, P = 0.02) and at 14 cM (CI = 59 cM) on BTA15 (1.11 ± 0.36, P <0.01). Finally, a QTL affecting MUFA content was found at 47 cM (CI = 59 cM) on BTA22 (1.85 ± 0.60, P = 0.02). To the best of our knowledge, this is the first report of QTL with dominance effects on the fatty acid composition of beef. Other results from this experiment (Alexander et al., 2007b), indicate QTL with additive effects on the quantities for saturated, mono-unsaturated and poly-unsaturated fatty acids in steaks from the Longissimus dorsi. However, only the QTL on BTA2 reported here were coincident with those previously reported.

Excess dietary saturated fatty acids are thought to predispose people to several cardiovascular diseases (e.g., Department of Health, 1994; Katan et al., 1994; 2000). Results of this study indicate that the relative amounts of SFA, MUFA, and PUFA are under a degree of genetic control. Thus, it may be possible to manipulate fatty acid composition using genetic markers and appropriate specialized crossbreeding systems in order to improve healthfulness of beef.

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