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# Gene expression patterns during intramuscular fat development in cattle[1]

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**ABSTRACT:** Deposition of intramuscular fat, or "marbling," in beef cattle contributes significantly to meat quality variables, including juiciness, flavor, and tenderness. The accumulation of intramuscular fat is largely influenced by the genetic background of cattle, as well as their age and nutrition. To identify genes that can be used as early biomarkers for the prediction of marbling capacity, we studied the muscle transcriptome of 2 cattle crossbreeds with contrasting intramuscular fat content. The transcriptomes of marbling LM tissue of heifers from Wagyu × Hereford (W×H; n = 6) and Piedmontese × Hereford (P×H; n = 7) crosses were profiled by using a combination of complementary DNA microarray and quantitative reverse transcription-PCR. Five biopsies of LM were taken from each animal at approximately 3, 7, 12, 20, and 25 mo from birth. Tissue was also collected from the LM of each animal at slaughter (approximately 30 mo). Microarray experiments, conducted on the first 3 biopsies of 2 animals from each crossbreed, identified 97 differentially expressed genes. The gene expression results indicated that the LM transcriptome of animals with high marbling potential (W×H) could be reliably distinguished from less marbled animals (P×H) when the animals were as young as 7 mo of age. At this early age, one cannot reliably determine meaningful differences in intramuscular fat deposition. We observed greater expression of a set of adipogenesis- and lipogenesis-related genes in the LM of young W×H animals compared with their P×H contemporaries. In contrast, genes highly expressed in P×H animals were associated with mitochondrial oxidative activity. Further quantitative reverse transcription-PCR experiments revealed that the messenger RNA of 6 of the lipogenesis-related genes also peaked at the age of 20 to 25 mo in W×H animals. The messenger RNA expression of *ADIPOQ*, *SCD*, and *THRSP* was highly correlated with intramuscular fat content of an individual in W×H animals. Our study provides clear evidence of early molecular changes associated with marbling and also identifies specific time frames when intramuscular fat development in cattle muscle can be detected by using gene expression. This information could be used by animal scientists to design optimal nutrition for high marbling potential. In addition, the genes found to be highly expressed during development of marbling could be used to develop genetic markers or biomarkers to assist with beef production strategies.

**Key words:** cattle, gene expression, intramuscular fat, marbling, microarray, muscle



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## INTRODUCTION

Intramuscular fat content, or "marbling," of cattle muscle is an important component of traits that influence eating quality, such as meat tenderness, juiciness, and taste (Hovenier et al., 1993). The accumulation of intramuscular fat is associated with the genetic background, development, and nutrition of an animal. The potential for cellular development of adipocytes is believed to be fixed relatively early in life and to change thereafter in either the size or number of cells that occur in proportion to the initial cell number and lipogenic proteins (Kirkland and Dobson, 1997; Caserta et al., 2001; Pethick et al., 2004). The importance of early life on intramuscular fat development suggests that it may be possible to predict adult intramuscular fat content by using biochemical measurements on muscle tissue in early life (Pethick et al., 2007).

A gene expression study of LM from Wagyu compared with Holstein animals at 11 mo of age found significantly greater adipogenesis- and lipogenesis-related gene expression in Wagyu cattle (Wang et al., 2005). However, when gene expression in the LM of newborn Wagyu × Hereford (**W×H**) calves was compared with the LM of newborn Piedmontese × Hereford (**P×H**), very few of the genes associated with adipogenesis were found to be significantly elevated (Lehnert et al., 2007). This suggests that the onset of marbling in animals occurs between birth and the early postweaning phase. However, the exact timing and development of marbling in cattle with a genetic disposition for this trait are still unknown.

To further address these issues, this study used a combination of microarray and quantitative reverse transcription-PCR (**qRT-PCR**) to measure the transcriptome of LM tissue of W×H and P×H cattle with contrasting amounts of intramuscular fat development at 6 consecutive time points between birth and 30 mo of age. This study demonstrated that the molecular changes associated with the processes of adipogenesis and lipogenesis set the 2 crossbreeds apart as early as 7 mo of age.

## MATERIALS AND METHODS

Use of animals and the procedures performed in this study were approved by the North Coast Animal Care and Ethics Committee.

### *Animals and RNA Samples*

Thirteen female progeny of 5 Wagyu (**W**; n = 6 progeny) sires and 5 Piedmontese (**P**; n = 7 progeny) sires and Hereford (**H**) dams were weaned at 7 to 8 mo of age and fed on improved temperate perennial pastures until feedlot entry at approximately 26 mo of age (Table 1). The animals were a subset of those well-nourished animals from the studies published by Cafe et al. (2006) and Greenwood et al. (2006). Animals were slaughtered at approximately 30 mo, when BW reached approximately 600 kg. The intramuscular fat percentage (**IMF%**; determined by near-infrared spectrophotometry) was determined as described by Perry et al. (2001). Five consecutive biopsy samples (approximately 5 g) were taken from the midlumbar region of the LM under local anesthetic when animals were aged 3 (**T3**), 7 (**T7**; weaning), 12 (**T12**), 20 (**T20**), and 25 (**T25**; feedlot entry) mo. These muscle biopsies were taken from alternate sides of the body of the animal to avoid previously biopsied sites. At slaughter (**T30**), approximately 20 g of LM tissue from each animal was also collected from the lumbar region of the LM, adjacent to the quartering site between the 12th and 13th ribs. Further details regarding the age and phenotype of experimental animals are listed in Table 1.

Total RNA was prepared by using Trizol reagent (Invitrogen, Carlsbad, CA), according to the instructions of the manufacturer. The RNA samples were treated with Turbo DNase I (Ambion, Austin TX), followed by a second DNase I treatment using RNAeasy Mini Kit columns (Qiagen, Valencia, CA). All purified total RNA samples were stored at −80°C for microarray experiments and qRT-PCR assays.

### *Microarray Experiment*

The RNA samples from 3 consecutive biopsy samples (T3, T7, and T12) of 4 animals were selected for the microarray experiment. Two W×H (animal identification no. 179 and 235) and 2 P×H (animal identification no. 130 and 285) crossbreeds were included in the study. These animals, identified with an asterisk (*) in Table 1, were selected because their extreme intramuscular fat performance within their breed contemporaries was expected to maximize the chances of identifying differentially expressed genes.

Total RNA (2 μg) was used for antisense RNA amplification according to the instructions of the manufacturer (MessageAmp kit, Ambion). Fluorescent labeling was performed on 5 μg of antisense RNA by using the indirect labeling method (Lehnert et al., 2004).

Twenty-one spotted complementary DNA (**cDNA**) microarray hybridizations containing 9,600 probes from muscle and fat tissue-derived cattle cDNA libraries on CMT GAPS II (Corning Inc., Lowell, MA) slides (Lehnert et al., 2004) were used for this experiment (Figure 1). The experimental layout was designed to allow a focus on the developmental aspect of the study, but also to permit a breed comparison to be carried out. It comprised a series of 12 hybridizations arranged in a multiple dye-swap to address the within-time, across-breed comparisons. Eight further hybridizations were arranged in an alternate dye sampling design layout to address the within-breed, across-time comparisons. Finally, a self-hybridization was performed and incorporated into the analysis as a measure of the pure error component. Fluorescently labeled cDNA were mixed in 50 μL of hybridization solution and hybridized under

**Table 1.** The identification (ID), genetic background, age (d) at each biopsy sampling time, and LM intramuscular fat percentage (IMF%) after slaughter for each experimental animal

| | | | Animal age (d) at each biopsy sampling time[4] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Animal ID[1] | Sire ID[2] | Dam[3] | 1st B (T3) | 2nd B (T7) | 3rd B (T12) | 4th B (T20) | 5th B (T25) | Slaughter (T30) | BW, kg before slaughter | LM IMF% at slaughter[5] |
| 58 | P 1 | H | 96 | 234 | 375 | 634 | 797 | 928 | 674 | 6.32 |
| 112 | P 1 | H | 85 | 223 | 364 | 623 | 786 | 917 | 542 | 4.25 |
| 130* | P 2 | H | 81 | 219 | 360 | 619 | 782 | 913 | 722 | 3.43 |
| 201 | P 3 | H | 67 | 205 | 346 | 605 | 768 | 899 | 614 | 6.05 |
| 224 | P 1 | H | 61 | 199 | 340 | 599 | 762 | 893 | 634 | 5.39 |
| 273 | P 4 | H | 47 | 185 | 326 | 585 | 748 | 880 | 708 | 8.16 |
| 285* | P 5 | H | 44 | 182 | 323 | 582 | 745 | 877 | 712 | 3.42 |
| Mean | | | 69 | 207 | 348 | 607 | 770 | 901 | 658 | 5.29 |
| SD | | | 19 | 19 | 19 | 19 | 19 | 19 | 65.5 | 1.73 |
| 99 | W 1 | H | 84 | 222 | 363 | 622 | 785 | 918 | 672 | 8.11 |
| 169 | W 1 | H | 75 | 213 | 354 | 613 | 776 | 907 | 698 | 8.38 |
| 179* | W 2 | H | 70 | 208 | 349 | 608 | 771 | 902 | 638 | 14.58 |
| 212 | W 3 | H | 63 | 201 | 342 | 601 | 764 | 895 | 708 | 8.00 |
| 235* | W 4 | H | 60 | 198 | 339 | 598 | 761 | 892 | 656 | 18.44 |
| 245 | W 5 | H | 56 | 194 | 335 | 594 | 757 | 888 | 690 | 6.80 |
| Mean | | | 68 | 206 | 347 | 606 | 769 | 900 | 677 | 10.72 |
| SD | | | 10 | 10 | 10 | 10 | 10 | 11 | 26.7 | 4.68 |

[1]Biopsies of the LM collected at T3, T7, and T12 from animals indicated by an asterisk (*) were used for microarray hybridization.
[2]Piedmontese sires, P1 to P5; Wagyu sires, W1 to W5.
[3]H = Hereford.
[4]Animal age (d) at biopsy (B). Biopsy time points are referred to as T3, T7, T12, T20, T25, and T30, corresponding to 3, 7, 12, 20, 25, and 30 mo of age.
[5]Intramuscular fat percentage as measured by near-infrared spectrophotometry of the carcass.

standard conditions (Lehnert et al., 2004). Microarray slides were scanned with the GenePix 4000A scanner (Molecular Devices, Sunnyvale, CA) at a resolution of 10 μm. The photomultiplier tube voltage was adjusted so the histograms of green (**Cy3**) and red (**Cy5**) channels overlapped. The intensity values for the Cy3 and Cy5 channels for each spot were acquired by GenePix-Pro 3.0 (Molecular Devices).

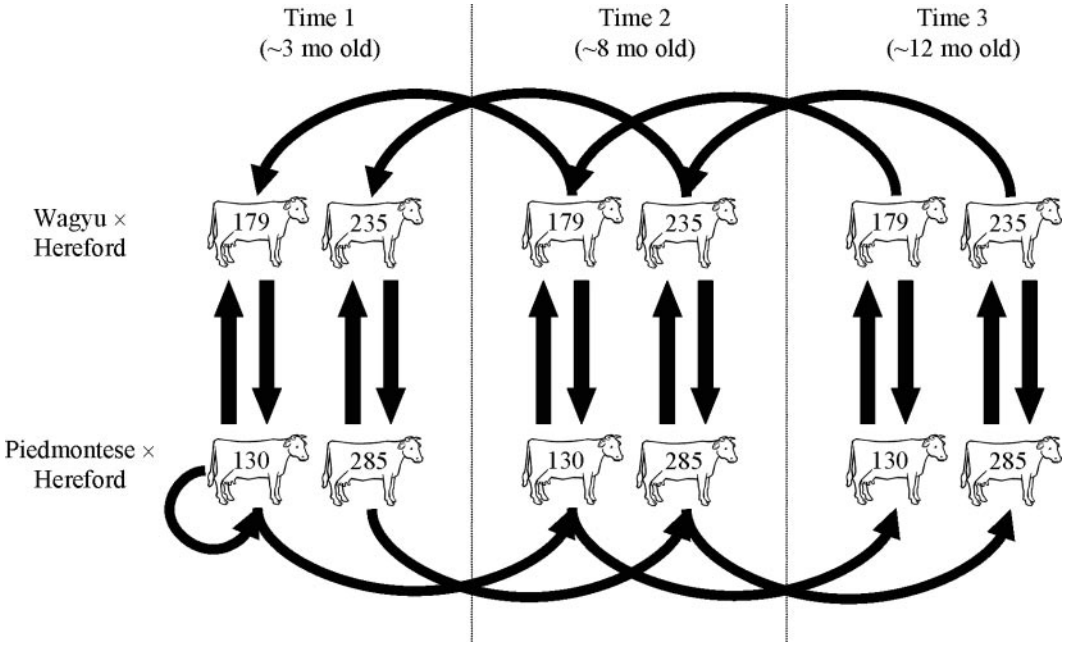


**Figure 1.** Microarray experimental design of LM transcription profiles. The experiment contained 21 slides and compared the expression profile in muscle tissue between 2 crossbreeds: Wagyu × Hereford and Piedmontese × Hereford, with 2 animals from each crossbreed, at 3 time points. The direction of the arrows indicates the labeling with either red or green dyes.

### *Microarray Data Analysis*

Gene expression intensity signals were subjected to a series of data acquisition criteria based on the signal-to-noise ratio and the mean-to-median correlation and as detailed in Tan et al. (2006). In brief, we used the following 2 editing criteria for data acquisition. First, we required that the signal-to-noise ratio (computed by dividing the background-corrected intensity by the SD of the background pixels) be greater than unity; second, we required that the correlation between the mean and the median signal intensities (computed by dividing the smaller of the mean or median by the larger) to be greater than 0.85. Tran et al. (2002) suggested not only that a correlation of 0.85 or greater retains more data than other methods, but also that the retained data are more accurate than traditional thresholds or common spot-flagging algorithms. However, these criteria were applied separately for the Cy5 and Cy3 intensity channels so that a different number of observations for each channel were obtained. These resulted in a total of 690,124 gene expression intensity readings (343,180 red and 346,944 green) on 8,129 genes (or probes or clones) that were background-corrected and base-2 log-transformed. The arithmetic mean and SD (in parentheses) for the red and green intensities were 11.10 (2.02) and 11.19 (1.97), respectively.

Data normalization was carried out by using a linear mixed ANOVA model and differentially expressed genes identified by model-based clustering via mixtures of distributions on the normalized expression of each gene at each breed and time point, as detailed in Reverter et al. (2004, 2005). In brief, the following linear mixed-effect model was fitted to the data:

$$Y_{ijktmn} = \mu + C_{ijk} + G_m + AG_{ijm} + DG_{km} + TG_{tm} + \varepsilon_{ijktmn},$$

where $Y_{ijktmn}$ represents the nth background-adjusted, normalized base-2 log intensity from the mth gene (probe) at the tth treatment (animal age and breed sample) from the ith array, jth printing block, and kth dye channel; C represents a comparison group fixed effect (2,016 levels), defined as those intensity measurements from the same array slide, printing block, and dye channel; G represent the random gene (probe) effects with 8,129 levels; AG, DG, and TG are the random interaction effects of array × gene, dye × gene, and treatment × gene, respectively; and ε is the random error term.

Variance components for random effects were estimated by using REML, and differentially expressed genes were identified after processing the appropriate linear combination of the BLUP of TG via model-based clustering, addressing the age as well as the breed comparison contrasts of interest.

A total of 5 contrasts were considered in the identification of differentially expressed genes. These included the 2 within-breed, across-time contrasts and the 3 within-time, across-breed contrasts. For each contrast, a 2-component normal mixture model was fitted, and posterior probabilities of belonging to the nonnull component, used to identify differentially expressed genes for an estimated experiment-wise false discovery rate of <1%, were computed as described by McLachlan et al. (2006).

### *qRT-PCR Assays*

To validate the microarray hybridization results, 11 genes were selected from the differentially expressed gene list for qRT-PCR assays. In addition, 5 adipogenesis-related genes were analyzed in the RNA samples by qRT-PCR. Primer sequences of these genes are listed in Table 2. The samples used in qRT-PCR assays were total RNA of all 6 time points from 13 animals (Table 1).

Single-stranded cDNA synthesis from 2 μg of total RNA, qRT-PCR assay, and data collection were performed as described previously (Wang et al., 2005). Sequence Detection Software (version 2.0; Applied Biosystems, Foster City, CA) results were exported as tab-delimited text files and imported into Microsoft Excel (Microsoft Corp., Redmond, WA) and Q-gene (Muller et al., 2002) for further analysis. The PCR efficiency for each primer pair was calculated by using the LinRegPCR program (Ramakers et al., 2003). The average amplification efficiency for each gene across all samples was used in Q-gene for all qRT-PCR analyses.

Four commonly used internal reference housekeeping (**HK**) genes, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), β-actin *(ACTB)*, 18S ribosomal RNA, and ribosomal protein large P0 (*RPLP0*) were tested for their suitability in this experiment; each gene was tested in a subset of samples (24 RNA samples from 2 W×H and 2 P×H at 6 time points) and analyzed by using GeNorm software (http://medgen31.ugent.be/jvdesomp/genorm/). The gene expression stability measure (**M**) is the average pair-wise variation for that gene with all other tested genes. The M values for all 4 HK genes were <0.5, which met the stability requirement to be an HK gene. The *RPLP0* gene showed the lowest M value and was therefore selected as the internal HK gene for data normalization (Vandesompele et al., 2002).

### *Correlation and Regression Analyses*

Importantly, qRT-PCR was performed on the 11 candidate genes not only to validate the differential expression observed in the microarray experiment, but also to evaluate the extent to which the expression of each gene was related to the intramuscular fat performance of the 13 cattle at each of the 6 time points. To this end, correlation analyses were performed to ascertain whether the expression of any of the individual genes was significantly related to the performance measures. Finally, the qRT-PCR mean normalized expression of

**Table 2.** Primer sequences used in quantitative reverse transcription-PCR assays

| Gene symbol | Gene name | Forward primer | Reverse primer |
|---|---|---|---|
| Differentially expressed genes from microarray analysis | | | |
| *ADIPOQ* | Adiponectin, C1Q and collagen domain containing | tcacaatggggtctatgcag | tgatgttcagaatcccctca |
| *FABP4* | Adipocyte-type fatty acid binding protein | cgtgggctttgctaccag | tggttgattttccatcccag |
| *SCD* | Stearoyl-CoA desaturase | ccagaggaggtactacaaacctg | agccaggtgacgttgagc |
| *DLK* | Delta-like 1 homolog | gcgtggtgaatggctcg | ggctgcaggtcttgtcca |
| *ANKRD1* | Ankyrin repeat domain 1 (cardiac muscle) | gacagaacctgtggatgtgc | acgattgccaaatgtccttc |
| *FN1* | Fibronectin | attgatgcaccatccaacct | cctggttccagaccagtgat |
| *COL1A1* | Collagen type I α I | tggtgacaagggtgagacag | gggagaccattgagtccatc |
| *COL1A2* | Collagen type I α 2 | ggtcgaagtggagagacagg | aggttcacccacagatccag |
| *COL3A1* | Collagen type 3 α I | | |
| *GAPDH* | Glyceraldehyde-3-phosphate dehydrogenase | cctggagaaacctgccaagt | agccgtattcattgtcatacca |
| *WNT10B* | Wingless-type MMTV integration site family, member 10B | ctgtaaccatgacatggacttcg | aggttttcagttaccacctgacg |
| Additional adipogenesis-related genes | | | |
| *CEBPB* | CCAAT/enhancer binding protein β | cgacagttgctccaccttct | ctcgcaggtcaagagcaag |
| *PPARG* | Peroxisome proliferator-activated receptor gamma | aaagcgtcagggttccacta | cccaaacctgatggcattat |
| *LPL* | Lipoprotein lipase | taccctgcctgaagtttccac | cccagtttcagccagactttc |
| *FAS* | Fatty acid synthesis | ggtgtggacatggtgacaga | acaatggcctcgtaggtgac |
| *THRSP* | Thyroid hormone responsive SPOT14 | aagaggctgaggaggagagc | ggactgccttctatcatgtgg |
| *SFRP5* | Secreted frizzled-related protein 5 | cctccagtgaccaagatctgtg | ttcttcatgtgcagcacgag |
| Housekeeping gene | | | |
| *RPLP0* | Acetic ribosomal protein large P0 | caaccctgaagtgcttgacat | aggcagatggatcagcca |

individual genes was further analyzed by fitting an ANOVA model that contained the main effects of breed and age and the covariate (regression) effect of IMF% nested within the breed × age interaction. These analyses were performed by using the procedures CORR and GLM (SAS Inst. Inc., Cary, NC).

## RESULTS

### *Detection of Differentially Expressed Genes by Microarray*

Ninety-seven genes, including 4 unannotated expression sequence tags, were identified as differentially expressed genes over 3 time points in the LM muscle of W×H compared with P×H (Table 3). They were classified into 9 categories according to their biological functions: adipogenesis and lipogenesis, energy metabolism, carbohydrate metabolism, oxidation, myogenesis and muscle development, extracellular structure, immune and stress responses, signaling, and transcription and translation. A further set of 31 differentially expressed genes were not associated with any of the above categories and were classified as "others." Figure 2 shows the number of differentially expressed genes in each category in W×H and P×H. Genes highly expressed in W×H animals were in the categories of adipogenesis and lipogenesis, extracellular structure, and signaling, whereas genes highly expressed in P×H animals dominated the categories of energy metabolism and oxidation.

### *Microarray Data Validation by qRT-PCR Assays*

Eleven differentially expressed genes from the microarray analysis were selected for qRT-PCR validation assays. Seven genes with likely roles in adipogenesis and lipogenesis and 4 genes coding for extracellular matrix components were chosen for validation by qRT-PCR. The assays were performed on biopsy samples of all 13 experimental animals at T3, T7, and T12. The variations between technical replicates were very small, whereas the variations between the biological replicates (animals) were large (data not shown). Overall, qRT-PCR expression profiles (as indicated by ratios of averaged expression in LM of W×H over P×H) for each selected gene closely mirrored those obtained from microarray assays (Table 4).

### *qRT-PCR Assays for 17 Selected Genes*

Eleven differentially expressed genes (Table 2) from microarray analysis and an additional 6 adipogenesis- and lipogenesis-related genes (*THRSP*, *FAS*, *LPL*, *CEBPB*, *PPARG*, and *SFRP5*) were measured for their mRNA expression in all 13 animals and across all 6 time points. At T7, significant differences were detected between W×H and P×H in 10 out of 17 genes (Figure 3). These genes include 2 adipose differentiation-associated genes (*CEBPB* and *PPARG*), 3 genes involved with fatty acid synthesis and metabolism (*ADIPOQ*, *FABP4*, and *FAS*), one gene from the wingless signal transduction pathway (*SFRP5*), and 4 connective tissue structure genes (*COL1A1, COL1A2, COL3A1*, and *FN1*). In addition, several genes were significantly different between the 2 crossbreeds at T25 and T30. In general, the adipogenesis- and lipogenesis-related genes were preferentially expressed in W×H animals at T7 and T25.

The mRNA expression for *CEBPB* were relatively greater at early time points, whereas *PPARG* displayed a slightly different expression profile, with a peak of

**Table 3.** Microarray identification of genes differentially expressed in LM of Wagyu × Hereford (W×H) compared with Piedmontese × Hereford (P×H) animals in at least 1 time point

| GenBank accession[1] | No. of array elements[2] | Gene symbol[3] | Gene name[3] | Microarray expression ratios of W:P[4] | | |
|---|---|---|---|---|---|---|
| | | | | T3 | T7 | T12 |
| Genes related to adipogenesis and lipogenesis | | | | | | |
| CF613470 | 32 | *FABP4* | Fatty acid-binding protein, adipocyte | 1.20 | 2.33 | 1.53 |
| CO729188 | 23 | *SCD* | Stearoyl-CoA desaturase (delta-9-desaturase) | 1.11 | 2.16 | 0.78 |
| CO729221 | 8 | *AdipQ* | Adipocyte, C1Q and collagen domain containing | 1.09 | 2.36 | 1.21 |
| CF613982 | 2 | *CLU* | Clusterin | 0.94 | 1.28 | 0.92 |
| BF773913 | 1 | *ACSM1* | Acyl-CoA synthetase medium-chain family member 1 | 0.87 | 1.32 | 0.92 |
| DW521722 | 1 | *APOE* | Apolipoprotein E | 0.59 | 0.53 | 0.50 |
| CF615136 | 1 | *SCARF1* | Scavenger receptor class F, member 1 isoform 1 precursor | 0.55 | 1.64 | 1.10 |
| Genes related to energy metabolism | | | | | | |
| CF615488 | 1 | *PPP1R3C* | Protein phosphatase 1, regulatory (inhibitor) subunit 3C | 2.46 | 0.68 | 0.89 |
| DW521731 | 2 | *ATP5J* | ATP synthase F0 subunit 6 | 1.85 | 0.59 | 0.38 |
| CF614094 | 1 | *PDK4* | Pyruvate dehydrogenase kinase, isoenzyme 4 | 1.06 | 2.71 | 1.96 |
| CF615087 | 2 | *PFKFB3* | 6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 | 0.87 | 2.72 | 1.31 |
| CF614549 | 1 | *CKM* | Creatine kinase, muscle | 0.83 | 0.66 | 0.57 |
| Gene related to carbohydrate metabolism | | | | | | |
| GAPDH_5′ | 2 | *GAPDH* | Glyceraldehyde-3-phosphate dehydrogenase | 0.48 | 0.87 | 1.12 |
| CF614691 | 1 | *ALDOA* | Aldolase A, fructose-bisphosphate | 0.41 | 0.52 | 0.59 |
| Genes related to oxidative metabolism | | | | | | |
| CF615544 | 1 | *UQCRB* | ubiquinol-cytochrome c reductase binding protein | 1.73 | 0.80 | 1.07 |
| CF613649 | 5 | *MTND3* | NADH dehydrogenase subunit 3 | 1.32 | 0.81 | 0.76 |
| CF613892 | 4 | *MTCYB* | Cytochrome *b* | 1.16 | 0.64 | 0.59 |
| DW521756 | 3 | *MTND5* | mitochondrion NADH dehydrogenase subunit 5 | 0.75 | 0.82 | 0.94 |
| CF615553 | 3 | *MTND4* | NADH dehydrogenase subunit 4 | 0.67 | 0.81 | 0.61 |
| CF615047 | 35 | *MTCO1* | Mitochondrially encoded cytochrome c oxidase I | 0.64 | 0.93 | 0.85 |
| DW521765 | 4 | *MTND4L* | NADH dehydrogenase subunit 4L | 0.57 | 0.80 | 0.57 |
| DW521773 | 1 | *COX7A2* | Cytochrome c oxidase subunit VIIa polypeptide 2 | 0.53 | 0.72 | 0.61 |
| DW521845 | 1 | *LDHA* | LDH-A mRNA for lactate dehydrogenase-A isozyme | 0.52 | 0.61 | 0.60 |
| Genes related to myogenesis and muscle development | | | | | | |
| CF614343 | 8 | *CSRP3* | Cysteine and glycine-rich protein 3 (cardiac LIM protein) | 2.05 | 0.89 | 1.37 |
| CF614523 | 8 | *CRYAB* | Crystallin, α B | 1.83 | 1.07 | 1.29 |
| CF615135 | 1 | *TPM2* | tropomyosin 2 (β) (TPM2), transcript variant 1 | 1.79 | 0.20 | 0.30 |
| CF614815 | 1 | *MYH1* | Myosin, heavy polypeptide 1, skeletal muscle | 0.74 | 0.97 | 0.62 |
| CF615306 | 8 | *ATP2A1* | ATPase, $Ca^{++}$ transporting, cardiac muscle, fast twitch 1 | 0.56 | 0.78 | 0.59 |
| CES014390 | 2 | *ACTB* | β-Actin_5′ | 0.52 | 1.04 | 0.85 |
| CF614690 | 1 | *TNN12* | Troponin I, skeletal, fast | 0.42 | 0.93 | 0.63 |
| CF614924 | 1 | *ACTN3* | Actinin, α 3 | 0.40 | 0.54 | 0.68 |
| CF614495 | 5 | *ACTN1* | Actin, α 1, skeletal muscle | 0.36 | 0.53 | 0.54 |
| Genes related to extra cellular structure | | | | | | |
| CO729170 | 4 | *FN1* | Fibronectin 1 (FN1), transcript variant 1 | 1.30 | 2.04 | 1.76 |
| CF613798 | 12 | *COL3A1* | Collagen, type III, α 1 | 1.17 | 1.32 | 1.77 |
| CF614144 | 2 | *MGP* | Matrix Gla protein | 1.09 | 1.93 | 1.55 |
| CF613952 | 28 | *COL1A1* | Collagen, type I, α 1 | 1.02 | 2.14 | 1.37 |
| DW521763 | 1 | *FMOD* | Fibromodulin | 0.90 | 1.66 | 0.71 |
| CF613956 | 10 | *COL1A2* | Collagen, type I, α 2 | 0.85 | 1.73 | 1.32 |
| DW521813 | 1 | *LAMA4* | Laminin α-4 chain precursor | 0.61 | 0.99 | 0.96 |
| Genes related to immune and stress responses | | | | | | |
| CF614108 | 5 | *HLA-A* | Major histocompatibility complex, class I, A precursor | 1.60 | 1.35 | 1.36 |
| DW521819 | 1 | *EPC1* | Enhancer of polycomb 1 | 1.39 | 1.16 | 1.73 |
| CF614214 | 4 | *HLA-B* | Major histocompatibility complex, class I, B | 1.29 | 1.04 | 1.33 |
| CF615362 | 2 | *HLA-C* | Major histocompatibility complex, class I, C | 1.26 | 1.23 | 1.45 |
| DW521838 | 1 | *PSMB5* | Proteasome (prosome, macropain) subunit, β type 5 | 1.19 | 1.31 | 1.32 |
| DW521638 | 5 | *TXNIP* | Thioredoxin interacting protein | 1.10 | 1.54 | 1.18 |
| DW521770 | 1 | *HLA-DQB1* | Major histocompatibility complex, class II, DQ β 1 | 0.88 | 0.70 | 0.50 |
| DW521762 | 1 | *RTN4* | Reticulon 4, transcript variant 3 | 0.84 | 0.60 | 0.49 |
| CES014034 | 1 | *CCL2* | Chemokine (C-C motif) ligand 2 | 0.69 | 0.82 | 0.84 |
| Genes related to signaling | | | | | | |
| DW521754 | 1 | *ASB5* | Ankyrin repeat and SOCS box-containing 5 | 2.75 | 1.25 | 0.92 |
| CF614403 | 6 | *ANKRD1* | Ankyrin repeat domain 1 (cardiac muscle) | 2.16 | 0.90 | 0.68 |
| CF614646 | 1 | *TRIM54* | Tripartite motif-containing 54 (TRIM54), transcript variant 2 | 2.03 | 0.72 | 0.77 |
| BF707278 | 1 | *ADRB2* | β-2 adrenergic receptor | 1.72 | 1.03 | 0.80 |
| DW521766 | 1 | *SH3BP4* | SH3-domain binding protein 4 | 1.46 | 1.99 | 1.47 |
| CF614111 | 5 | *IGFBP5* | Insulin-like growth factor binding protein 5 | 1.31 | 0.81 | 0.59 |

*Continued*

**Table 3 (Continued).** Microarray identification of genes differentially expressed in LM of Wagyu × Hereford (W×H) compared with Piedmontese × Hereford (P×H) animals in at least 1 time point

| GenBank accession[1] | No. of array elements[2] | Gene symbol[3] | Gene name[3] | Microarray expression ratios of W:P[4] | | |
|---|---|---|---|---|---|---|
| | | | | T3 | T7 | T12 |
| CF614035 | 2 | *SPARC* | Secreted protein, acidic, cysteine-rich (osteonectin) | 1.21 | 1.41 | 1.27 |
| CF614138 | 1 | *IGFBP5* | Insulin-like growth factor-binding protein 5 | 1.11 | 0.64 | 0.40 |
| DW521760 | 1 | *RGS2* | Regulator of G-protein signaling 2, 24kDa | 0.94 | 1.32 | 1.18 |
| BE683284 | 1 | *WNT10B* | Wingless type 10b (wnt10b) homolog | 0.86 | 1.23 | 2.04 |
| AW289395 | 1 | *STAT1* | Signal transducer and activator of transcription 1 | 0.43 | 1.08 | 0.82 |
| Genes related to transcription and translation | | | | | | |
| CO729196 | 1 | *HSPB1* | Heat shock 27kDa protein 1 | 2.16 | 0.93 | 1.49 |
| CF613588 | 1 | *FOS* | v-fos FBJ murine osteosarcoma viral oncogene homolog | 2.06 | 1.51 | 2.66 |
| DW521833 | 1 | *UBA52* | Ubiquitin A-52 residue ribosomal protein fusion product 1 | 1.53 | 0.95 | 1.53 |
| DW521761 | 1 | *MEF2C* | Myocyte enhancer factor 2C | 1.41 | 0.86 | 0.66 |
| CF613993 | 1 | *RPL14* | Ribosomal protein L14 | 1.32 | 2.27 | 1.01 |
| CF615612 | 7 | *ATF4* | Activating transcription factor 4 | 0.56 | 0.81 | 0.76 |
| Others | | | | | | |
| CF615310 | 4 | *HSPA1A* | Heat shock 70kDa protein 1A | 2.33 | 0.94 | 1.23 |
| DW521842 | 1 | *SETBP1* | SET binding protein 1 | 2.11 | 0.91 | 0.86 |
| CO729198 | 3 | *KBTBD5* | Kelch repeat and BTB (POZ) domain containing 5 | 2.03 | 0.83 | 0.83 |
| DW521700 | 4 | *MUSTN1* | Musculoskeletal, embryonic nuclear protein 1 | 1.97 | 1.12 | 1.26 |
| CO729185 | 1 | *HSPB8* | Heat shock 22kDa protein 8 | 1.82 | 1.15 | 1.17 |
| BF606016 | 2 | *DLK* | Delta-like 1 homolog | 1.77 | 1.23 | 0.90 |
| CO729222 | 1 | *MAP1A* | Microtubule-associated protein 1A | 1.62 | 0.85 | 0.88 |
| DW521839 | 1 | *TOMM22* | Translocase of outer mitochondrial membrane 22 homolog (yeast) | 1.60 | 1.14 | 1.25 |
| DW521758 | 1 | *EST* | Similar to *Schistosoma japonicum* SJCHGC09300 protein | 1.27 | 0.85 | 1.61 |
| DW521830 | 1 | *CSTB* | Cystatin B (stefin B) | 1.01 | 1.49 | 0.70 |
| CF615380 | 1 | *DDIT4* | DNA-damage-inducible transcript 4 | 0.97 | 0.91 | 1.74 |
| DW521843 | 1 | *SYNE2* | Spectrin repeat containing, nuclear envelope 2 | 0.97 | 0.66 | 0.56 |
| CO729200 | 1 | *SQSTM1* | Sequestosome 1 | 0.93 | 2.17 | 0.84 |
| DW521834 | 1 | *EST* | *Bos taurus* hypothetical protein LOC784639 | 0.85 | 1.46 | 0.90 |
| DW521768 | 1 | *LARP5* | La ribonucleoprotein domain family, member 5 | 0.78 | 1.83 | 1.49 |
| DW521822 | 1 | *EST* | *Bos taurus* hypothetical protein LOC783730 | 0.78 | 1.08 | 1.66 |
| DW521764 | 1 | *PDZRN3* | PDZ domain containing RING finger 3 | 0.73 | 0.69 | 0.70 |
| CF614142 | 3 | *MTRNR1* | Mitochondrially encoded 12S RNA | 0.72 | 0.46 | 1.31 |
| CF615327 | 1 | *GSTM3* | Glutathione S-transferase M3 (brain) | 0.69 | 0.51 | 0.53 |
| DW521769 | 1 | *FNTB* | Farnesyltransferase, CAAX box, β | 0.68 | 0.79 | 0.49 |
| DW521846 | 1 | *UBC* | Polyubiquitin, transcript variant 27 | 0.67 | 0.61 | 0.59 |
| DW521759 | 2 | *FHL1* | Four and a half LIM domains 1 | 0.58 | 1.01 | 0.94 |
| DW521831 | 1 | *tRNA-Pro* | Mitochondrion tRNA-Pro | 0.57 | 0.62 | 0.62 |
| DW521836 | 1 | *CA3* | Carbonic anhydrase III | 0.55 | 0.72 | 0.66 |
| DW521757 | 1 | *MLF1* | Myeloid leukemia factor 1 | 0.55 | 0.68 | 0.92 |
| CF614734 | 1 | *CCNG1* | Cyclin G1 (CCNG1), transcript variant 1 | 0.51 | 0.69 | 0.50 |
| DW521837 | 1 | *EST* | *Bos taurus* hypothetical LOC518370 | 0.49 | 0.60 | 0.43 |
| DW521829 | 1 | *AHNAK* | Nucleoprotein isoform 1 | 0.44 | 0.61 | 0.66 |
| CF614846 | 1 | *C11orf39* | Chromosome 11 open reading frame 39 | 0.26 | 0.53 | 0.63 |
| DW521840 | 1 | *H3F3A* | H3 histone, family 3A | 0.26 | 0.33 | 0.16 |
| DW521835 | 1 | *CTDSP1* | CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1 | 0.21 | 0.46 | 0.25 |

[1]GenBank identifier for differentially expressed gene. In cases where multiple array elements were identified for the same gene, one GenBank Accession identification was selected as representative.

[2]Number of array elements representing each differentially expressed gene.

[3]Genes or gene products for which our microarray clones showed a match at the nucleotide level. The BLASTn results (Altschul et al., 1990) used for annotation had sequence homologies with scores >97.6 and E (expect) values >2.00E-20.

[4]Ratios of expression intensity values ($\log_2$ value) of W×H over P×H samples at the 3 biopsy time points. Where multiple array elements were identified for the same gene, the mean of the Wagyu (W):Piedmontese (P) expression ratio is listed. The gene lists in each functional category are ranked according to the most significant difference of expression between W and P at the T3 time point (biopsy at 3 mo of age).

expression occurring at T25 and T30 for W×H animals. This expression peak was not observed in P×H animals.

The *WNT10B* mRNA began with greater expression in P×H animals at T3 and reached its peak at T25 (Figure 3). In contrast, *SFRP5* showed decreased expression in both breeds until T30, when *SFRP5* reached its peak expression in W×H animals only.

Coordinated gene expression changes were observed for 4 connective tissue-related genes, *COL1A1*, *CO-*

**Table 4.** Comparison of gene expression measurements by microarray and quantitative reverse transcription PCR (qRT-PCR) at T3, T7, and T12[1]

| | T3 | | T7 | | T12 | |
|---|---|---|---|---|---|---|
| Gene | Microarray[2] | qRT-PCR[3] | Microarray[2] | qRT-PCR[3] | Microarray[2] | qRT-PCR[3] |
| *ADIPOQ* | 1.09 | 0.98 | 2.36 | 3.69 | 1.21 | 0.74 |
| *FABP4* | 1.20 | 1.01 | 2.33 | 2.68 | 1.53 | 0.63 |
| *SCD* | 1.11 | 1.03 | 2.16 | 2.08 | 0.78 | 0.53 |
| *DLK* | 1.77 | 1.34 | 1.23 | 1.99 | 0.90 | 1.30 |
| *GAPDH* | 0.48 | 0.86 | 0.87 | 0.95 | 1.12 | 0.99 |
| *WNT10B* | 0.86 | 0.52 | 1.23 | 0.79 | 2.04 | 1.70 |
| *ANKRD1* | 2.16 | 1.44 | 0.90 | 0.61 | 0.68 | 0.89 |
| *FN1* | 1.30 | 0.69 | 2.04 | 1.87 | 1.76 | 1.68 |
| *COL1A1* | 1.02 | 1.47 | 2.14 | 1.73 | 1.37 | 1.76 |
| *COL1A2* | 0.85 | 0.89 | 1.73 | 1.42 | 1.32 | 0.94 |
| *COL3A1* | 1.17 | 1.60 | 1.32 | 1.34 | 1.77 | 1.64 |
| Correlation[4] | 0.511 | 0.511 | 0.807** | 0.807** | 0.634* | 0.634* |

[1]Biopsy time points are referred to as T3, T7, and T12, corresponding to 3, 7, and 12 mo of age.
[2]Microarray Wagyu × Hereford (W×H) and Piedmontese × Hereford (P×H) fold changes are taken from Table 2.
[3]Ratios of gene expression measurements in LM samples from W×H over P×H animals by qRT-PCR.
[4]Significant correlations were indicated as $*P < 0.05$ and $**P < 0.01$.

*L1A2*, *COL3A1*, and *FN1*. The mRNA abundance was generally greater in W×H animals, for which peaks of expression occurred at T7 and T25. The expression profiles closely mirrored those of the lipid metabolism genes (Figure 3).

### *Correlation Between mRNA of Adipogenic- and Lipogenic-Related Genes and Intramuscular Fat Content*

Strong positive correlations were observed between mRNA expression of several adipogenic- and lipogenic-related genes and IMF% in W×H animals (Table 5). The *ADIPOQ, SCD*, *THRSP*, and *FAS* genes showed particularly strong correlations with IMF% at both T20 and T25. In contrast, the correlations between gene expression and IMF% in P×H animals were not as strong as in W×H animals, except at T3, when strong correlations were seen for *SCD* and *FAS*. Figure 4 illustrates the correlation, at T20 and T25, between *ADIPOQ* mRNA expression and IMF%, with $R^2 = 0.94$ and 0.85 in W×H animals compared with $R^2 = 0.09$ and 0.01 in P×H animals. The ANOVA model for *ADIPOQ* containing the main effects of breed and age and the covariate (regression) effect of IMF% nested within the breed × age interaction explained 55.7% of the variation in *ADIPO*. Importantly, the only regression coefficients of *ADIPOQ* on IMF% found to be significantly different from zero ($P < 0.001$) were for the W×H cross at the later ages of T20 (with regression estimated at 0.019 ± 0.006), T25 (estimated at 0.018 ± 0.006), and T30 (estimated at 0.027 ± 0.006). No strong correlations were observed between gene expression of adipogenesis- and lipogenesis-related genes and rump fat in both W×H and P×H animals (data not shown).

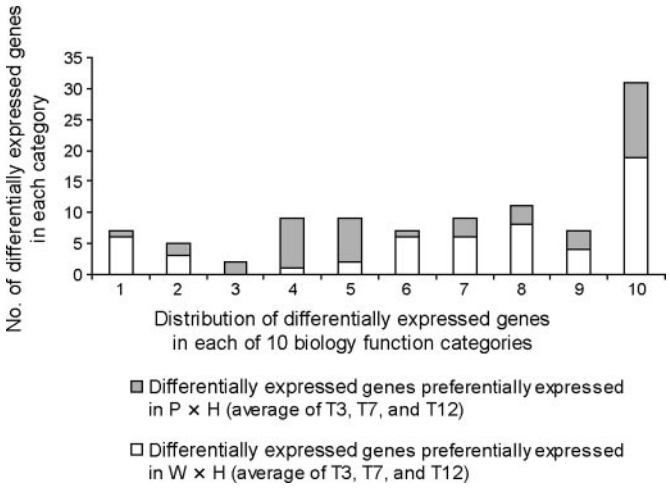


**Figure 2.** A number of differentially expressed genes highly expressed in Wagyu × Hereford (W×H) animals (unfilled boxes) and highly expressed in Piedmontese × Hereford (P×H) animals (filled boxes) were classified into 10 categories according to their biological functions: (1) adipogenesis and lipogenesis; (2) energy metabolism; (3) carbohydrate metabolism; (4) oxidation; (5) myogenesis and muscle development; (6) extracellular structure; (7) immune and stress; (8) signaling; (9) transcription and translation; and (10) others. Biopsy time points are referred to as T3, T7, and T12, corresponding to 3, 7, and 12 mo of age.

## DISCUSSION

### *Experimental Design and Analysis Considerations*

To maximize the chances of identifying differentially expressed genes in the microarray component of this study, the 2 cattle with the most extreme performance, in terms of intramuscular fat deposition, within each sire breed group were selected. In the qRT-PCR validation of the study, RNA samples from all 13 cattle and across all 6 time points were used to obtain an unbiased estimate of the expression of candidate genes. To allow

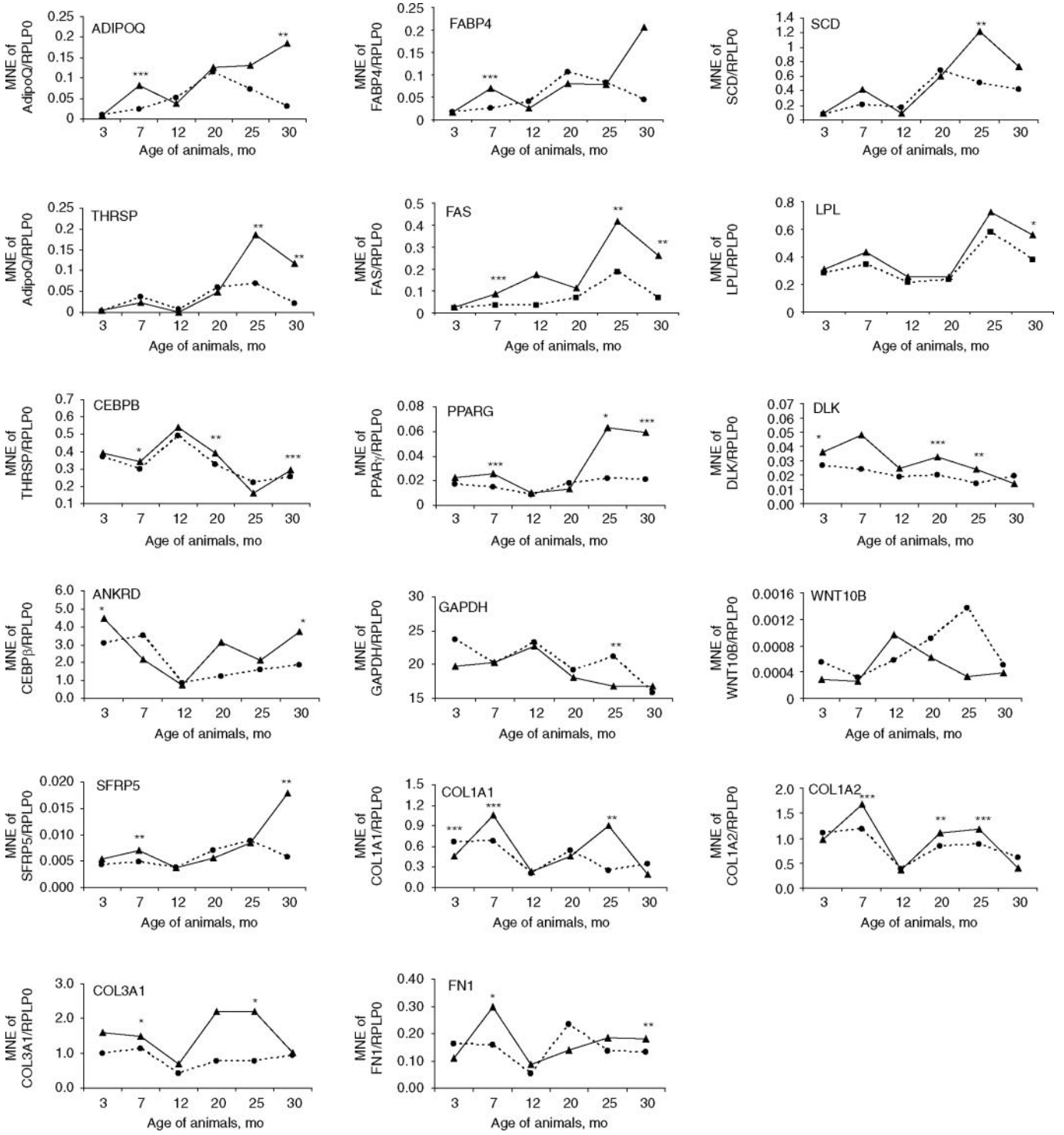


**Figure 3.** Mean normalized expression (MNE) of 17 genes (identified in Table 2) in LM of Wagyu × Hereford (W×H; n = 6, solid line) and Piedmontese × Hereford (P×H; n = 7, dashed line) at 6 time points. The MNE expression values were established by quantitative reverse transcription-PCR assays normalized to ribosomal protein large P0 (*RPLP0*) expression measured at the same time. The statistical significance at any given time point between the 2 crossbreeds was analyzed by unpaired *t*-test (*$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$).

us to distinguish expression changes within and across sire genotype, sire breed was fitted in the statistical models for the analysis of both the microarray and the qRT-PCR data. Statistical inference was performed at the level of both between and within breeds. The resulting normalized mean expression of each gene within each breed was used for building the between-breed contrast W vs. P in the differential expression analyses for microarray and RT-PCR, respectively, as well as for assessing the strength of the relationship between the expression of a gene and intramuscular fat deposition within breed.

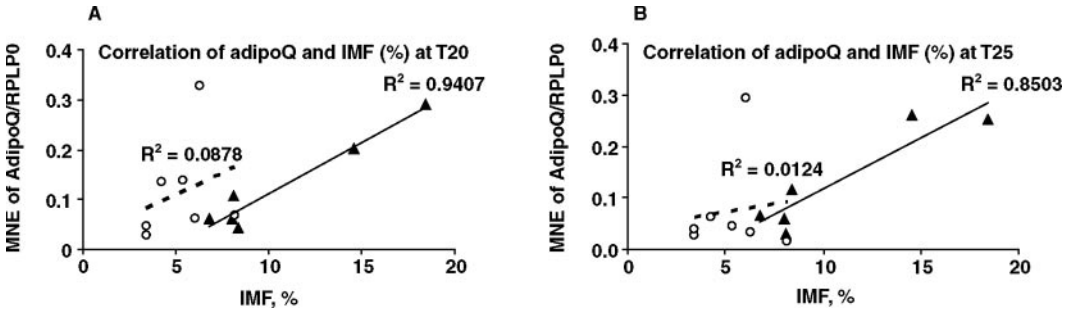


**Figure 4.** Correlations between mean normalized expression (MNE) of adiponectin, C1Q, and collagen domain-containing (*ADIPOQ*) gene expression at (A) T20 (biopsy time point corresponding to 20 mo of age) and (B) T25 (biopsy time point corresponding to 25 mo of age) and intramuscular fat (IMF) percentage in LM tissue of Wagyu × Hereford (n = 6, solid line) and Piedmontese × Hereford (n = 7, dashed line).

### *Early Development of Intramuscular Fat in W×H Animals*

Intramuscular fat deposition is associated with genetic background, as well as the development and nutrition of an animal (Pethick et al., 2004). Wagyu animals, such as Japanese Black, are genetically predisposed to deposit intramuscular fat. Previous microarray studies indicated a time frame during which the development of adipogenesis and lipogenesis may occur in W animals (Wang et al., 2005; Lehnert et al., 2007). In the present study, gene expression profiling of LM from W×H and P×H crossbreeds was carried out retrospectively, in biopsies that had been collected before substantial intramuscular fat deposition took place. Examination of the amounts of gene transcripts at these early stages provides an opportunity to observe the window of time during muscle development when transcriptional activation occurs.

One transcription factor involved in the preadipocyte differentiation process, *CEBPB*, was seen as differentially expressed between the 2 crossbreeds as early as T7. This may indicate that the predisposition of the animal to develop fat and activate one of the core early transcription factors is well developed by that time for W×H animals. It is interesting to note, however, that *CEBPB* and *PPARG* showed opposite expression patterns in W×H animals: *CEBPB* was expressed at relatively greater abundance at earlier time points, whereas *PPARG* expression increased only at the last 2 time points, during the accumulation of fat deposits. This gene expression pattern is consistent with the established model of adipogenesis, in which *CEBPB* is required for subsequent induction of *PPARG* expression (Wu et al., 1995; Clarke et al., 1997). Although *PPARG* was found to be elevated only in the later time points, the mRNA expression of several adipogenic- and lipogenic-related genes, *ADIPOQ*, *FABP4*, and *FAS*, was markedly elevated in W×H animals at T7, when animals were close to weaning. This early separation between the 2 crossbreeds provides molecular evidence for the early cellular development of adipocytes in animals with marbling potential. This evidence points to a time frame

**Table 5.** Correlations between gene expression and intramuscular fat percentage in LM samples at 6 time points[1]

| Gene | Breed[2] | T3 | T7 | T12 | T20 | T25 | T30 |
|---|---|---|---|---|---|---|---|
| *ADIPOQ* | W×H | −0.50 | 0.13 | 0.65 | 0.97*** | 0.92** | 0.66 |
| | P×H | −0.06 | 0.44 | 0.10 | 0.30 | 0.11 | −0.07 |
| *FABP4* | W×H | −0.29 | −0.09 | 0.20 | 0.95 | 0.83* | 0.63 |
| | P×H | 0.04 | −0.13 | 0.17 | 0.21 | 0.14 | −0.57 |
| *SCD* | W×H | −0.43 | −0.03 | −0.34 | 0.77 | 0.92** | 0.54 |
| | P×H | 0.84* | −0.01 | 0.19 | 0.25 | −0.01 | 0.26 |
| *THRSP* | W×H | −0.17 | 0.08 | −0.35 | 0.94** | 0.10 | 0.22 |
| | P×H | −0.26 | 0.46 | −0.25 | −0.07 | 0.59 | −0.57 |
| *FAS* | W×H | −0.73 | 0.23 | −0.29 | 0.94** | 0.83* | 0.55 |
| | P×H | 0.92** | 0.60 | 0.35 | 0.34 | 0.18 | 0.71 |
| *LPL* | W×H | −0.55 | −0.20 | −0.28 | 0.30 | 0.53 | 0.61 |
| | P×H | 0.25 | −0.30 | 0.03 | 0.25 | −0.09 | −0.36 |

[1]Gene expression values were mean normalized expression values established by using quantitative reverse transcription-PCR and normalized against *RPLP0* expression. The intramuscular fat percentage was determined by near-infrared spectrophotometry. Significant correlations were indicated as $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Biopsy time points are referred to as T3, T7, T12, T20, T25, and T30, corresponding to 3, 7, 12, 20, 25, and 30 mo of age. Genes identified in Table 2.

[2]Crossbreds used in this experiment are Wagyu × Hereford (W×H) and Piedmontese × Hereford (P×H).

during which early invention is possible to maximize intramuscular fat in later life.

### *Peak Expression for Lipogenic Genes in W× H Animals at T25*

A peak of expression for many of the adipogenesis- and lipogenesis-associated genes was detected by using qRT-PCR assays at T25, before the animals entered the feedlot. This expression pattern confirms findings from a previous study (Wang et al., 2005). It is a commonly held view that intramuscular fat is an adipose tissue depot that is laid down only in the mature animal (Pethick et al., 2004). Our data support the contention that at 25 mo of age, W×H animals had reached this stage of maturity and were depositing or beginning to deposit substantial amounts of intramuscular fat. In contrast, by 30 mo, P×H animals were not depositing substantial amounts of intramuscular fat. However, our data were unable to exclude the possibility that intramuscular fat accumulation in the P×H crossbreed may have commenced at a later stage in the life of the animal.

The mRNA expression of adipogenic- and lipogenic-related genes in LM at T25 had strong positive correlations with IMF% measured after slaughter. This evidence indicated that a snapshot of mRNA abundance at T25 could be used to predict the development of intramuscular fat during the subsequent feedlot phase. Hence, it offers a potential application of using gene expression data to predict the marbling trait.

### *Expression of Mitochondrial Genes*

There is evidence of coordinate gene expression differences between W×H and P×H animals. Mitochondrial genes, such as *MTCYB*, *COX7A2*, *MTND4*, and *MTND4L*, are more highly expressed in P×H animals. These genes are the subunits of the respiratory complexes that are involved in mitochondrial oxidative phosphorylation. Because mitochondrial oxidative phosphorylation is involved in the generation of energy, such as ATP, these findings indicate that compared with the W×H, the P×H animals may use more energy to support more rapid growth of muscle during the period to weaning, when fractional rates of muscle growth are increased, as we have discussed previously (Lehnert et al., 2007). In this regard, it has also been shown that animals sired by increased muscle growth genotypes have greater oxidative enzyme activity in muscles, including LM, than those sired by decreased muscle growth genotypes during early postnatal life, despite a shift toward more glycolytic myofiber types in the offspring of sire genotypes with greater muscle growth (Gardner et al., 2007; Greenwood et al., 2007; Warner et al., 2007). Animals with an increased basal energy requirement may also be expected to store less triacylglycerol in adipose tissue.

### *Expression of Connective Tissue Structure Genes*

It is interesting to note that, in the highly marbling W×H animals, the expression of several extracellular protein genes loosely mirrored that of adipogenic-related genes. Four connective tissue protein genes (*COL1A1*, *COL1A2*, *CIL3A1*, and *FN1*) and 2 genes that influence the synthesis and interaction with the extracellular matrix (*SPARC* and *FMOD*) showed this pattern. Intramuscular fat has been shown to develop within the perimysium connective tissue alongside myofibers (Moody and Cassens, 1968). Perimysium is a major connective tissue in muscle and contains collagen fibers, as well as other minor components. This perhaps suggests that the expansion of the extracellular matrix may be a prerequisite for intramuscular fat development. This conclusion is further strengthened by previous reports showing that intramuscular connective tissue undergoes structural changes during the fattening of Japanese Black cattle (Nishimura et al., 1999) and that type XII collagen isoforms are expressed during bovine adipogenesis (Tahara et al., 2004).

### *Wingless Signaling Pathway and Marbling Development*

In a previous study (Tan et al., 2006), we showed that the wingless signaling pathway may play a role during bovine adipogenesis. The *WNT10B* gene is a molecular switch that governs 3T3-L1 adipogenesis and maintains preadipocytes in an undifferentiated state. Interruption of *WNT10B* expression in preadipocyte cells will lead to adipogenic differentiation (Ross et al., 2000). When the expression pattern of *WNT10B* and the gene coding for its binding partner, *SFRP5*, are examined in LM samples, it becomes apparent that when the expression of *WNT10B* decreases, *SFRP5* increases. These results strengthen the argument that adipogenesis and intramuscular fat deposition in cattle may be regulated in part by the wingless signaling pathway, and that tissue-specific manipulation of this pathway could potentially be used to increase intramuscular fat deposition.

In conclusion, we have provided molecular evidence of early intramuscular adipogenesis. The coordinate expression pattern of a set of adipogenesis- and lipogenesis-related genes and their strong positive correlation with intramuscular fat content at slaughter provide potential for the development of markers for predicting marbling. Gene expression profiling provides an effective tool to discover gene expression changes associated with production traits and to discover genes contributing to quantitative variation between breeds of farm animals.

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