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Beef quality with different intramuscular fat content and proteomic analysis using isobaric tag for relative and absolute quantitation of differentially expressed proteins

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ABSTRACT

Intramuscular fat (IMF) is an important trait for beef eating quality. The mechanism of how IMF is deposited in beef cattle muscle is not clear at the molecular level. The muscle (*M. longissimus lumborum*: LL) of a group of Xiangxi yellow × Angus cattle with high fat levels (HF), was compared to the muscle of a low fat group (LF). The meat quality and the expressed protein patterns were compared. It was shown that LL from the HF animals had a greater fat content ($P < 0.05$) and lower moisture content ($P < 0.05$) than LL from LF animals. Forty seven sarcoplasmic proteins were differentially expressed and identified between the two groups. These proteins are involved in 6 molecular functions and 16 biological processes, and affect the Mitogen-activated protein kinases pathway, insulin pathway and c-Jun N-terminal kinases leading to greater IMF deposition. Cattle in the HF group had greater oxidative capacity and lower glycolytic levels suggesting a greater energetic efficiency.

1. Introduction

The deposition of intramuscular fat (IMF), which is termed marbling when visually assessed, is a key factor influencing the eating quality of beef (Corbin et al., 2015; Ron et al., 2015). Sensory acceptability of beef increases as marbling increases as judged by consumers in a number of countries (e.g. China: Liang et al., 2016, Japan: Okumura et al., 2007, United States: Corbin et al., 2015). The deposition of IMF varies considerably between individual animals reflecting differences in production systems and genetics (Pethick, Harper, & Oddy, 2004). Given the cost of intensively feeding cattle to improve IMF levels there is sound logic for understanding the variation in deposition.

Several approaches are being used to study IMF deposition such as the gene chip (Clark et al., 2011) which is being used to understand the genetic control of the process or by studying gene polymorphisms (Liu, Tian, Zan, Wang, & Cui, 2010; Seong, Suh, Park, Lee, & Kong, 2012). Another approach is to examine vascular endothelial growth factor expression patterns and IMF deposition capability (Yamada, Kawakami, & Nakanishi, 2009; Yamada & Nakanishi, 2012).

Genes transcribe mRNA, leading to the production of proteins (Lippolis & Reinhardt, 2008), and these proteins perform physiological functions indicative of biological activity. Proteomics has been used in meat science to explore the basis of meat variation in tenderness (Bjarnadottir et al., 2012), color (Canto et al., 2015), water holding capability and so on (Almeida et al., 2015). There has been limited research using proteomics to understand the important proteins impacting on beef IMF (Kim et al., 2008; Zhang et al., 2010) and how fat deposition is regulated that is not clear. There is a need to further define the bovine adipogenesis-specific proteins and the mechanisms of their activity (Romao, He, McAllister, & Guan, 2014). Isobaric tag for relative and absolute quantitation (iTRAQ) is a novel and accurate proteomics technique for identifying and quantifying the abundance changes of multiple (up to eight) distinct protein samples (Boehm, Putz, Altenhofer, Sickmann, & Falk, 2007; Ding et al., 2013; Wiese, Reidegeld, Meyer, & Warscheid, 2007). This technique was introduced to meat science and applied to beef tenderness research recently, and it was shown that proteins identified by both iTRAQ and 2-DE exhibit similar changes (Bjarnadottir et al., 2012), which suggested iTRAQ has potential as a new technique for exploring variation in meat traits.

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On this basis a study was conducted to compare the physicochemical and eating quality of meat from cattle with high IMF levels against those with low IMF levels and to identify the differentially expressed proteins involved in bovine intramuscular fat (IMF) deposition. The hypothesis was that muscle from the two diverse groups of cattle would exhibit differential expression of proteins that regulate intramuscular fat deposition and as a first step this would provide a theoretical basis for predicting the intramuscular fat deposition ability of individual animals more accurately.

2. Materials and methods

2.1. Animals, carcass sampling, measurement and experimental design

Seventy-eight (78) Xiangxi yellow × Angus cattle (58 castrated bulls and 20 cows), were all born at the Tianhua Animal Husbandry Corporation and allotted to individual pens at 18 months of age. All animals were given the same diet, housing and management and slaughtered at 30 months of age using standard processes at the abattoir of the Tianhua Animal Husbandry Corporation. Before the day of slaughter, the animals were transported to an abattoir and fasted for approximately 24 h with access to water. The experiment was undertaken following the guidelines outlined by the Animal Ethics Committee at Shandong Agricultural University and all experimental procedures were approved by the State Scientific and Technological Commission (China, 19881114).

On the day of slaughter, the animals were weighed (body weight W1), stunned by captive bolt pistol at the X-crossing point of horns and eyes, hung and bled. About 10 g of muscle sample (*M. longissimus lumborum*; LL) was taken from the 12th–13th rib area 45 min postmortem from each left half carcass and frozen in liquid nitrogen. These muscle samples were stored in a cryogenic refrigerator (−80 °C) until analysis. The hot carcass weight (W2) was recorded and the dressing percentage was calculated using W1 and W2 (dressing percentage = W2/W1 × 100).

The carcasses were chilled at 4 °C and after chilling for 4 days, the back fat thickness at the 12th–13th ribs was measured using calipers and then two sections of the LL were removed from each carcass for measurement of shear force, and three slices of 1 cm thick were prepared (all subcutaneous fat was removed) for the determination of fat and moisture content. All samples were vacuum packaged and stored at −20 °C until tested.

In order to eliminate sex differences, the present study just selected 12 castrated bulls with large differences in IMF content of the LL for further analysis. Samples from 6 animals (numbered with 1, 2, 3, 4, 5, 6) with the greatest fat content were selected for detailed examination (HF) and samples from another 6 animals (designated A, B, C, D, E, F) with the lowest fat content were selected (LF).

2.2. Temperature and pH measurement

Carcass temperature and pH values were measured at 45 min and 24 h post mortem using a digital thermometer (DM6801A, Shenzhen Victor Hi-tech Co. Ltd., China) and a portable pH meter (SenvenGo, Mettler-Toledo, Switzerland). The pH meter was calibrated in chilled buffers at pH 4.00 and 7.00. The probes were inserted into the center (approximately 3 cm deep) of the LL samples between the 12th–13th ribs. Each carcass was measured three times at each time and the average value used for analysis.

2.3. Intramuscular fat and moisture content determination

The IMF content was analyzed on ground samples of muscle according to the procedures described by the (AOAC, 1990). Moisture content was determined by drying ground samples (5 g, 3 replicates) to a constant weight at 105 °C for 24 h, and the moisture content calculated using before and after drying weights.

2.4. Cooking loss and Warner–Bratzler shear force

The samples for Warner–Bratzler shear force (WBSF) testing were thawed in a chiller (2 ± 2.0 °C) overnight and cooked individually in plastic bags in a water bath at 79 ± 1.0 °C. During cooking, the central temperature of each sample was tracked using a digital thermometer until the sample internal temperature reached 70 °C (AquaTuff™ 351, Cooper–Atkins Corporation, USA). Then, the cooked beef samples were stored in a chiller (2 ± 2.0 °C) overnight. The weight of the beef samples was recorded before cooking, after cooking and storage to calculate percentage cooking loss. Cooking loss = (sample weight before cooking − sample weight after weight) / sample weight before cooking × 100%. Nine to twelve cores (1.27 cm in diameter) parallel to the muscle fiber orientation were removed from each sample and analyzed for WBSF. Cores were sheared once using a texture analysis analyzer (Model TA-XT2i Stable Micro System, England) with a HDP/BSW blade. The WBSF value was calculated as the average readings for cores from the same sample.

2.5. Proteomics analysis

2.5.1. Protein extraction

Muscle samples (0.2 g) were chopped on ice, then homogenized with 1 ml 8 M urea and a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, Missouri, USA), and then subjected to ultrasonication (Scientz-CV18, China) for 5 min (Ultrasonic treatment 2 s with pause 2 s, 22% power) on ice. The homogenate was then centrifuged at 10,000*g* for 10 min at 4 °C, and the supernatant used to determine the sarcoplasmic protein concentration using the Bradford assay.

2.5.2. Protein denaturation, reduction, and alkylation

A sample of protein (75 μg) for each animal was reduced using 10 mM dithiothreitol for 60 min at 56 °C in water bath, and then alkylated by 55 mM iodoacetamide for 60 min at room temperature in the dark. Quadruple pre-cooled acetone was added to each sample for 3 h and precipitated at −20 °C, and then centrifuged at 20,000 g for 20 min at 4 °C. The sediments were redissolved in 50% triethylammonium bicarbonate (TEAB) and 0.1% sodium dodecyl sulfate using 3 min of ultrasonic treatment to assist dissolution. The protein sample was finally digested using trypsin at a mass ratio of 3.3:100 enzyme/protein for 6 h at 37 °C, then replenished with trypsin and digested for a further 2 h at 37 °C. The digestion liquid was freeze-dried using Thermo SPD2010 (Thermo Fisher Scientific, USA) and redissolved using 30 μl TEAB (water: TEAB = 1:1). Finally, the protein concentration was determined using a 2-D Quant Kit (GE Healthcare, USA) for labeling.

2.5.3. iTRAQ labeling of the protein digest

Muscle samples from the 12 individual cattles were analyzed in two iTRAQ runs (First, Second). Each iTRAQ run included eight muscle protein samples. The samples designated 1, 2, 3, 4, A, B, C and D were tested in the first run and the samples designated 3, 4, 5, 6, C, D, E and F were tested in the second run. Replicates of 3, 4, C, and D were tested in both runs to evaluate reproducibility.

Each iTRAQ reagent vial (Applied Biosystems, USA) and samples were equilibrated to room temperature. And then 50 μl ethyl alcohol was added to each iTRAQ reagent vial, vibrated for 1 min and centrifuged. Protein (100 μg) from each sample was mixed with iTRAQ reagents according to the design described above and let stand for 2 h at room temperature, and then dried under vacuum using Thermo SPD2010 (Thermo Fisher Scientific, USA).