Development of FQ-PCR method to determine the level of *ADD1* expression in fatty and lean pigs

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ABSTRACT. To determine how *adipocyte determination and differentiation factor 1* (*ADD1*), a gene involved in the determination of pork quality, is regulated in Laiwu and Large pigs, we used TaqMan fluorescence quantitative real-time polymerase chain reaction (FQ-PCR) to detect differential expression in the longissimus muscle of Laiwu (fatty) and Large White (lean) pigs. In this study, the ADD1 and GAPDH cDNA sequences were cloned using a T-A cloning assay, and the clone sequences were consistent with those deposited in GenBank. Thus, the target fragment was successfully recombined into the vector, and its integrity was maintained. The standard curve and regression equation were established through the optimized FQ-PCR protocol. The standard curve of porcine ADD1 and GAPDH cDNA was determined, and its linear range extension could reach seven orders of magnitudes. The results showed that this method was used to quantify *ADD1* expression in the longissimus muscle of two breeds of pig, and was found to be accurate, sensitive, and convenient. These

results provide information regarding porcine *ADD1* mRNA expression and the mechanism of adipocyte differentiation, and this study could help in the effort to meet the demands of consumers interested in the maintenance of health and prevention of obesity. Furthermore, it could lead to new approaches in the prevention and clinical treatment of this disease.

Key words: Pig; ADD1; FQ-PCR; TaqMan fluorogenic probe