## Effect of energy metabolism and proteolysis on the toughness of intermediate ultimate pH beef

<u>Yan Zhao</u><sup>1</sup>, Xiao Kong<sup>1</sup>, Xiaoyin Yang<sup>1</sup>, Lixian Zhu<sup>1</sup>, Rongrong Liang<sup>1</sup>, Xin Luo<sup>1</sup>, Liang Zhang<sup>1</sup>, David L Hopkins<sup>1,2</sup>, Yanwei Mao<sup>1\*</sup>, Yimin Zhang<sup>1\*</sup>

<sup>1</sup> College of Food Science and Engineering, Shandong Agricultural University, Taian, China

<sup>2</sup> NSW Department of Primary Industries, Centre for Red Meat and Sheep Development, PO Box 129, Cowra NSW 2794, Australia

**Introduction:** At present, the energy metabolism characteristics of different pHu beef in the early postmortem period are still unclear. Targeted metabolomics can help us understand the dynamic changes of energy metabolites in beef within the first 24 h postmortem when the beef has different ultimate pH's. This approach will enable the identification of key metabolites, and thus reveal the toughness development mechanism of intermediate pHu beef.

**Materials and Methods:** A total of 40 Simmental crossbred bulls were randomly selected in a commercial abattoir. About 30 g samples were collected from the left side of Longissmus lumborum (LL) at 0.75, 3, 10 and 24 h postmortem and immediately frozen in liquid nitrogen. Muscle pH was measured between the 12/13th ribs using a portable pH meter at 24 h postmortem. According to the ultimate pH, the carcasses were divided into three groups: normal pHu (pH = 5.4 - 5.8), intermediate pHu (pH = 5.8 - 6.2) and high pHu (pH > 6.2). Metabolites were extracted, and separated using an Agilent 1290 Infinity LC ultra-high performance liquid chromatography system coupled to a 5500 QTRAP mass spectrometer (AB SCIEX, USA). All data were analyzed using Multiquant<sup>TM</sup> software (AB SCIEX, USA) to extract the chromatographic peak area and retention time. Spearman Rank Correlation between tenderness and energy metabolites was applied using IBM SPSS Statistics (Version 22.0, SPSS Inc., USA). At 48 h postmortem, the LL were removed from the left side of 15 carcasses (5 for each pH group), and each LL was cut into 2.54 cm thick steaks and randomly assigned to 5 ageing time points (0, 3, 7, 14 and 21 d). WBSF (Warner-Bratzler shear force) was determined during aging period. Data difference of shear force between the treatments were analyzed by the MIXED procedure using SAS (Institute Inc., Cary, NC, Version 9.2.), ageing time, pHu group and their interactions were fixed factors, while carcass was the random factor, the significant difference was considered at P < 0.05.

**Results:** In this study, it was found that intermediate pHu group exhibited higher WBSF than the other two groups with the extension of aging time. A total of 24 metabolites were detected and the interaction of pHu group and postmortem time had a significant effect on the content of 4 metabolites, including NADH, ADP, ATP and GTP, P < 0.05. The pHu group had the main effects on the content of 17 metabolites. Among them, eight metabolites exhibited a higher concentration in the normal pHu group (succinate, aconitate, pyruvate, lactate, D-G6P,  $\beta$ -D-F6P, NAD+ and cyclic-AMP), three were higher in the intermediate pHu group (GMP, AMP and citrate), while six were higher in the high pHu group (FMN, oxaloacetate, malate, fumarate, GTP and TPP). We conducted Spearman correlation analysis on shear force and the content of succinic acid, lactic acid, aconitonic acid, GMP, citric acid and D-G6P at 24 h postmortem, and negatively correlated with the contents of NADH, GDP and FMN. While, the contents of GMP and citric acid were higher in the intermediate pHu group, this also showed that the higher GMP and citric acid contents in the intermediate pHu group had an effect on its tenderness. In addition, the content of NADH in the intermediate pHu group had an effect on its poor tenderness.

**Conclusion:** Within the first 24 h postmortem, the formation of pHu were closely related to the metabolites involved in the glycolytic pathway. The meat toughness in the intermediate pHu group was also closely related to energy metabolism, such that the higher GMP and citric acid content and the lower NADH content can adversely affect its tenderness.