UNIVERSITY of GUELPH

ONTARIO AGRICULTURAL COLLEGE

DEPARTMENT OF ANIMAL BIOSCIENCES

PhD Defence

Effects of essential amino acids on transcription factors and cell activities by primary bovine mammary epithelial cells

Boning Li

Date: July 5th 2023 at 9:00am

The PhD Defence for Boning Li has been scheduled for July 5th, 2024 at 9:00am. The defence will be held online via Teams and in room 141: https://teams.microsoft.com/l/meetup-join/19%3ameeting_NjljOTllYjAtZjg1Mi00MDQ1LTk5ZGMtMDg1ZWEyZTczZjEx%40thread.v2/0?context=%7b%22Tid%22%3a%22be62a12b-2cad-49a1-a5fa-85f4f3156a7d%22%2c%22Oid%22%3a%22fbd28915-dda5-478f-8ecb-a3682dcf0c3a%22%7d

The exam committee will consist of:

Examining Chair: Dr. Marcio Duarte

Advisor: Dr. John Cant

Advisory Committee Member: Dr. John Doelman

Additional Committee Member: Dr. Eduardo Ribeiro

External Examiner Member: Dr. Xin Zhao

Abstract:

Essential amino acids (EAA) regulate protein synthesis in mammary epithelial cells by rapidly altering the phosphorylation state of translation factors. However, the long-term transcriptional response to EAA supply has not been well understood. The objectives of this thesis were to: 1) determine if and when cell activities and expression of selected transcription factors were affected under EAA deficiency, 2) explore differentially expressed genes and upstream transcription regulators under EAA deficiency using an RNA-sequencing method, 3) determine the signaling pathway of activating transcription factor 4 (ATF4) under EAA deficiency using knockdown techniques. All experiments were performed in primary cultures of bovine mammary epithelial cells after differentiation with Histidine (His), Lysine (Lys), or Methionine (Met) deficiency. It was found that all EAA deficiency treatments decreased the rates of protein synthesis and cell proliferation but did not affect lipogenesis in cells after 24 h. Multiple transcription factors that had the functions related to the stress response (ATF4), and cell proliferation, apoptosis, and autophagy, (forkhead box protein M1 FOXM1 and nuclear protein 1 NUPR1), were involved in the amino acid deficiency response. RNA-sequencing results revealed that EAA deficiency affected signaling pathways related to cell turnover and interferon. Knockdown of ATF4 affected protein synthesis, cell proliferation, apoptosis, and autophagy by regulating its downstream target genes or by forming heterodimers with other transcriptional factor in bovine mammary epithelial cells. These results have confirmed that EAA deficiency affects protein synthesis in mammary epithelial cells not only through translational regulation but also through transcriptional regulation via transcription factors.