UNIVERSITY of GUELPH

ONTARIO AGRICULTURAL COLLEGE

DEPARTMENT OF ANIMAL BIOSCIENCES

MSc. Defence

Characterization of Processive Endoglucanase Stability *in Vitro* as Exogenous Fibre Biocatalysts in Pig Nutrition

Laurence Cheng

Date: September 30th, 2021 at 11:00am

The MSc Defence for Laurence Cheng has been scheduled for Thursday September 30th 2021 at 11:00am. The defence will be held online via Teams: https://teams.microsoft.com/l/meetup-join/19%3ameeting_OWNiYTQ5YmItNGEzNC00ZWFkLTkxNjItNDIxNjJmZDY1YTM0%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. Julang Li

Advisor: Dr. Ming Fan

Adv. Committee Member: Dr. Elijah Kiarie

Additional Member: Dr. Kate Shoveller

Abstract:

Poor efficiency of dietary fibre utilization limits pork production profit margins and environmental sustainability. This thesis research was to investigate stability of two processive endoglucanases, referred to as GH5-tCel5A1 and GH5-p4818Cel5_2A and overexpressed in the ClearColi® BL21 (DE3) cell. Three-dimensional models predicted presence of cysteine residues on the cellulases' catalytic sites; and results of *in vitro* time course experiments further shown these cellulases were susceptible to auto-oxidation by air-borne O_2 and were unstable. *In vitro* cellulases' stability was also examined under the mimicked porcine gastric and the small intestinal conditions. Eadie-Hofstee inhibition kinetic analyses shown that both cellulases lost significant levels of their initial enzyme activities within 2 – 3 h of incubations and were thus not stable under the porcine gastrointestinal environmental conditions. It is concluded that enzyme protein engineering and alternatively post-fermentation enzyme processing need to be further pursued to enable both cellulases as potentially efficacious exogenous fibre enzymes.