FUNCTION SITE OF SUPPLEMENTAL ESCHERICHIA COLI PHYTASE IN THE GASTROINTESTINAL TRACTS AND ITS ROLE IN BONE METABOLISM OF YOUNG PIGS

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Angela Rose Pagano

August 2006

ABSTRACT

This thesis consists of two studies on phytase. The objective of the first study was to determine the functional site of a supplemental *Escherichia coli* AppA2 phytase and its impact on digesta phosphorus and calcium concentrations in different segments of the gastrointestinal tract of pigs. In Exp. 1, 18 weanling pigs [8.3 ± 0.2 kg body weight (BW)] were allotted to three groups (n = 6) and fed a low-P (0.4%) corn-soy basal diet (BD), BD + phytase [500 units (U)/kg] or BD + inorganic P (0.1%) for 4 wk. In Exp. 2, 30 weanling pigs (14.5 ± 0.2 kg BW) were allotted to three groups (n = 10) and fed BD, BD + phytase (500 U/kg) or BD + phytase (2000 U/kg) for 2 wk. Five or six pigs out of each treatment group were slaughtered at the end of both trials to collect digesta from six segments of the digestive tract to assay for phytase activity, soluble P concentration, and(or) total P and Ca concentrations. Pigs fed BD + phytase had similar phytase activities in the stomach, duodenum, and upper jejunum digesta, and the detected activities were proportional to the supplemental levels of dietary AppA2. But, no phytase activity was detected in digesta of these three segments from pigs fed BD or BD + 0.1% iP or in digesta of lower jejunum and ileum from any of the treatment groups. Digesta soluble P decreased sharply from the stomach to duodenum of pigs fed BD + phytase or BD + iP, whereas it peaked in the upper jejunum of pigs fed BD. Colonic digesta phytase activity and soluble P were highest ($P < 0.05$) in pigs fed only BD and were inversely affected by dietary phytase supplementation. Pigs fed BD + phytase showed phytase-dose dependent reductions ($P < 0.05$) in total colonic P and (or) Ca concentrations, compared with those fed BD or BD + iP. In conclusion, supplemental dietary AppA2 mainly functioned in the stomach, but remained fairly active in the upper jejunum. Colonic microbial phytase activity was greatly reduced by

the supplemental phytase-mediated phytate-phosphorus hydrolysis in the fore segments of the digestive tract via substrate limitation.

The objective of the second study was to determine if high levels of supplemental dietary microbial phytase, in a phosphorus (P)-adequate diet, additionally improved bone strength of growing female pigs. For three experiments a total of 80 pigs (28-35 d old) were fed a low-P (0.4%) corn-soy basal diet (BD), or BD + 0.2% or 0.25% inorganic P and (or) 1,000 or 2,000 units *E. coli* AppA2 phytase/kg for 4-6 wk. At the end of the experiments, pigs were slaughtered to collect 3rd and 4th metacarpals from front legs to test for bone strength. In Exp. 3, metacarpals were analyzed for their contents of Ca, P and other minerals. Weekly growth performance, plasma alkaline phosphatase activity and plasma inorganic P, were improved by phytase and inorganic P in BD. While these measures were not further improved by supplementing phytase at 2,000 U/kg in the P-adequate diets, bone strength of pigs fed the additional phytase was 12% ($P < 0.05$) greater than that of those fed only the P-adequate diets. The additional 2,000 U/kg in the P-adequate diets did not affect bone concentrations of Ca, P, K, Na, S, Mg, Fe, Mn, Zn, B or Cr, but resulted in 7% ($P < 0.05$) increase in bone Sr concentration. In conclusion, supplementing phytase at 2,000 U/kg of the P-adequate diets produced additional benefit on bone strength of young female pigs, and the improvement was not directly associated with P accretion.

In summary, the two studies reported in this thesis help in locating the functional site of *E. coli* AppA2 phytase in the digestive tract of young pigs, in unveiling a novel role of high levels of AppA2 activity in bone metabolism of young pigs.

BIOGRAPHICAL SKETCH

Angela R. Pagano was born in Ogdensburg, NY. She graduated salutatorian from Heuvelton Central School in June of 1998. Angela attended Hamilton College, located in Clinton, NY, and graduated with a BA in Biology and French Literature in May 2002. She came to Cornell University to pursue her MS in Animal Science after an internship as an environmental educator and a position with SCA/Americorps monitoring Blanding's turtles.

This thesis is dedicated to my parents, Joseph and Lynne Pagano, who have provided me with much love and support throughout the years.

ACKNOWLEDGMENTS

Foremost, I would like to thank Dr. Xingen Lei for his guidance; I have achieved much more than I would have thought possible coming into my Masters program. I appreciate Dr. Dennis Miller and Dr. Austic for their constructive input to this thesis. I would also like to thank Dr. Wilson Pond for his wisdom, Dr. Taewan Kim for his guidance in understanding phytase, Koji Yasuda for all his help, Carol Roneker for her tremendous assistance in the lab, and Joanne Parsons for her general support during the past two years. I also would like to thank Debbie Ross, Michael Rutzke and Dr. James Bartsch for making mineral and bone strength analyses possible. In addition, I extend my gratitude to Jeremy Weaver, Weipeng Mu, Michael Scimeca, Erin Peterson, and Steve Kirby for their help. Furthermore, I would like to thank Karl Roneker, without his sweat and hard work these studies would not have been possible.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES